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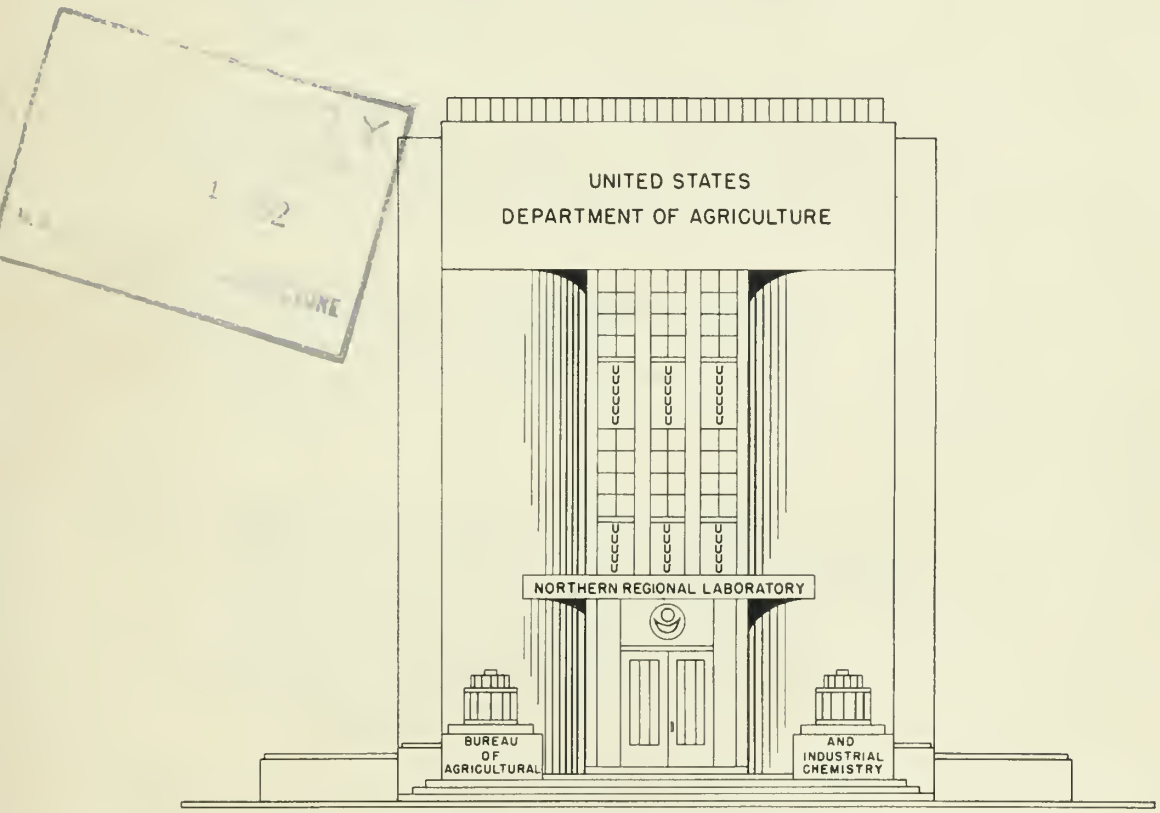
SELECTED METHODS FOR DETERMINING REDUCING SUGARS



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SELECTED METHODS FOR DETERMINING REDUCING SUGARS

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INTRODUCTION

This manual has been compiled to bring together detailed procedures for the methods selected by the Starch and Dextrose Division of the Northern Regional Research Laboratory for the determination of reducing sugars. The methods chosen, some of the operational details, and the units in which the results are expressed reflect the interests and physical facilities of the Division; however, these details do not prevent general use of the methods in other laboratories. The procedures are given in sufficient detail, with tables, so that analyses can be made without recourse to other texts, although the literature references given will afford a ready means for obtaining background and additional information.

With so many reducing sugar methods available, the question often arises as to which method is best adapted to a specific purpose. In the introduction to each method the scope and application is stated. Consideration is given to the nature and purity of the samples, the accuracy required, the number of determinations to be made, and the experience of the worker who will use the method. The Munson and Walker copper reduction method has the widest applicability, high precision and accuracy, and tables for the method have been well established; yet it is not well suited for routine analyses when many concurrent determinations must be made. The Munson and Walker method with the Shaffer and Hartmann determination of reduced copper without filtration allows faster operation with but slightly less accuracy and precision. A similar method by which somewhat smaller amounts of reducing sugar can be measured is that of Schoorl. Other variations of the Munson and Walker method require close attention to details to obtain accurate results. The method of Lane and Eynon as given in this manual is restricted to the determination of the dextrose equivalent of sirups. It is an accurate method in which the skill of the analyst is a very important factor. Results by the Sichert and Bleyer method for "true" dextrose must be accepted with reservations, since the specificity of the copper acetate reagent for monoses is not absolute. When the sample size is limited to a few milligrams of reducing sugar, the Somogyi micro copper method should be used. The

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application of the Somogyi method to the differential determination of glucose, maltose, and reducing dextrins in hydrolysis mixtures has been of particular value.

Ferricyanide methods are somewhat more precise, rapid, and convenient than copper methods and are more reliable in the hands of inexperienced workers. However, alkaline ferricyanide is less specific as an oxidant for sugars than alkaline copper complexes, and the range of sample size for published methods is much less than that of the Munson and Walker method.

Iodometric (hypoiodite) methods are nearly specific for aldoses, and pure aldoses can be determined with high precision and accuracy; yet many substances other than aldoses react with hypoiodite or iodine and limit the applicability. Iodometric methods are used chiefly for the determination of glucose, maltose, or lactose in the presence of sucrose or ketoses. Recently, an iodometric method has been used for the micro-estimation of sugars and methylated sugars separated on paper chromatograms.

References cited (italic numbers in parentheses) are numbered consecutively for each method to which they apply and are grouped at the end of the description of that method. The explanatory notes cited in the text will be found at the end of each procedure. Dextrose and glucose as used in this manual refer to D-glucose, regardless of commercial usage of the terms. All temperatures are centigrade.

Mention of firm names or proprietary products is made solely for information; it does not imply endorsement of such firms or products over other firms engaged in similar business or over products of similar nature not mentioned.

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I. COPPER METHODS

A. MUNSON AND WALKER UNIFIED METHOD

Introduction

The method of Munson and Walker *(1)* was designed to apply to all reducing sugars, thus unifying the many methods previously in use which were generally designed for individual sugars and offered no basis for comparisons among different sugars. The unified procedure soon became quite generally used, especially in the United States.

This simple method for total reducing power may be used with any sample containing reducing carbohydrates in sufficient amounts. The results are reproducible, and existing tables *(2,3c,4,5,6)* permit interpretation in terms of several sugars. Tables for other sugars can be prepared as needed without changing the procedure.

The procedure consists of oxidizing the sample by heating in a specified manner with a standard, freshly mixed, alkaline cupric tartrate solution. The precipitated cuprous oxide is determined by filtering, dissolving in ferric sulfate solution, and titrating with potassium permanganate to measure the amount of ferrous sulfate produced.

Reagents

Soxhlet solution "A" (cupric sulfate).-69.28 gm. of pure copper sulfate crystals, $CuSO_4 \cdot 5H_2O$, per liter. This solution should be checked for copper content by the method of Appendix I-C, since the crystals are often low in copper.

Soxhlet solution "B" (alkaline tartrate).-346 gm. of Rochelle salt, $KNaC_4H_4O_6 \cdot 4H_2O$, and 100 gm. of carbonate-free sodium hydroxide (note 12) per liter. Exact composition of this solution is less critical than that of "A".

Ferric sulfate solution.- 55 gm. of anhydrous ferric sulfate, $Fe_2(SO_4)_3$, or 135 gm. of ferric ammonium alum, $FeNH_4(SO_4)_2 \cdot 12H_2O$, per liter. The iron content of the salt used should be checked in advance by strong ignition to Fe_2O_3.

Potassium permanganate solution, 0.15 N.-A solution containing 4.98 gm. of potassium permanganate per liter is aged for several days, filtered through asbestos or sintered glass and standardized against arsenious oxide as described in Appendix I-B.

Sulfuric acid solution.- Approximately 4 *N*. 109 ml. concentrated sulfuric acid per liter.

Procedure

The sample chosen shall be of such size that its reducing power will equal between 5 and 235 mg. of glucose, preferably in the upper half of this range. The sample is dissolved (note 1) and placed in a 400-ml. Pyrex beaker (Griffin form) with a pinch of talc to prevent bumping and sufficient water to bring the total volume to 50±1 ml. A volumetric pipet is used to add 25.00 ml. of Soxhlet solution "A", after which 25 ml. of Soxhlet solution "B" is added (note 2) from a graduated cylinder.

The beaker is then covered with a watch glass and set on an electric heater so adjusted (notes 3 and 4) that the solution will start boiling in just 4 minutes (notes 5 and 6). After a total heating time of exactly 6 minutes (note 7) the beaker is removed from the heater and the reaction mixture immediately filtered on an asbestos mat in a Gooch crucible. Complete transfer of cuprous oxide to the crucible is unnecessary, but both beaker and crucible contents should be thoroughly washed with water.

The mat and precipitate from the crucible are transferred to the beaker, using a glass rod, and adhering cuprous oxide is washed from the crucible with the aid of some of the ferric sulfate solution (note 8). A total of about 50 ml. of ferric sulfate solution is added from a graduated cylinder or dispensing pipet and the mixture is thoroughly stirred until no more red particles of cuprous oxide may be seen through the bottom of the beaker (note 9).

The solution is acidified with 20 ml. of 4 *N* sulfuric acid (from a graduate) and immediately titrated with the standard permanganate solution. A 50 ml. buret contains sufficient 0.15 *N* permanganate for samples producing a maximum amount of cuprous oxide (note 10). The end point is reached when the pink color of permanganate, the last ml. of which must be added dropwise, persists for 30 seconds after the last drop has been added to the previously colorless solution.

A blank is run, covering the entire procedure and using all reagent. The titer of the blank is usually below 0.5 ml.

The reactions involved in determining reduced copper are:

$$Cu_2O + Fe_2(SO_4)_3 \longrightarrow 2\ FeSO_4 + CuSO_4 \cdot CuO$$

$$10FeSO_4 + 2KMnO_4 + 8H_2SO_4 \longrightarrow 5Fe_2(SO_4)_3 + K_2SO_4 + 2MnSO_4 + 8H_2O$$

The reducing power of the sample is first calculated in terms of the amount of copper reduced from the cupric to the cuprous state, thus:

$$(\text{Sample titer} - \text{blank titer}) \times \frac{\text{Normality of permanganate}}{0.01573\ \text{m.e./mg. Cu}} = \text{mg. Cu.}$$

The amount of sugar (dextrose unless otherwise specified) represented by this amount of copper is read from the table of Appendix II-A, or from tables (note 11) which have been published for less common sugars *(4)*. This amount of sugar (in mg.) is divided by the weight of sample (in gm.) to give the usual expression of reducing power as equivalent to so many milligrams of sugar (usually glucose) per gram.

Sample calculation: If the permanganate is 0.1566 *N*, sample wt. 0.2017 gm., sample titer 15.73 ml., and blank titer 0.19 ml., then

$$(15.73 - 0.19) \times \frac{0.1566}{0.01573} = 154.7\ \text{mg. copper.}$$

Hammond's table shows 154.7 mg. Cu $\rightleftharpoons$ 77.1 mg. dextrose; hence, the reducing power of the sample is

$$\frac{77.1}{0.2017} = 392\ \text{mg. of dextrose per gm.}$$

Notes

1. Samples not soluble in water may be dissolved in a little alcohol, which should also be used in the blank determination.

2. Solution "A" may be added in advance, but "B" should not be put in until just before heating. Soxhlet solutions when combined are known as Fehling solution.

3. Precision Scientific Co. Type RH heater, 550 watt, rheostat control, exposed heating element, ceramic "nest" for beaker.

4. Trial runs with 50 ml. of water, some talc, and the usual volumes of Soxhlet solutions are used to adjust the heater, which should be turned on at least 30 minutes before use so that it may reach equilibrium.

5. A steady stream of bubbles breaking the surface is considered "boiling."

6. A tolerance of ±15 seconds is usually satisfactory. Hammond *(2,3a)* held to 4 minutes ±5 seconds

7. Usually timed by a mechanical or electric timer with warning bell. Such timers should be checked against a good stop watch.

8. Avoid adding water. Diluting the ferric sulfate solution retards its action on the cuprous oxide.

9. The difficulty with which large quantities of cuprous oxide dissolve in neutral ferric sulfate solution is a principal disadvantage of this method.

10. Samples of low reducing power (below 25 mg. of dextrose) may be titrated more accurately with a standard .033 *N* permanganate solution.

11. For use of tables based on Cu_2O instead of Cu, multiply milligrams of copper by 1.1258 to get milligrams of cuprous oxide

12. Dissolve 100 gm. of C.P. NaOH pellets in 110 ml. water. When cool, filter through sintered glass, titrate a portion and take a volume equivalent to 100 gm. of NaOH.

Discussion

In the Starch and Dextrose Division of this Laboratory the method has been applied chiefly as an arbitrary measure of reducing power expressed as milligrams of dextrose per gram. The method is reliable if the procedure is carefully followed with special attention to having the copper sulfate solution at proper strength, the permanganate accurately standardized, and heating conditions as specified. In the last-named case, the exact total time of heating and prompt filtration to stop reaction are most essential.

The reproducibility should be within 0.4 percent in the higher range and within 0.2 mg. (of dextrose) in the lower ranges for most samples. Accuracy is about equally good if the sample consists of a single compound or mixture for which tables have been made, but mixing two or more reducing sugars or having a non-reducing carbohydrate present distorts the results, as may be seen by comparing the various columns of Hammond's table *(2,3c)*. Thus, work depending on Munson-Walker determinations before and after reactions which eliminate one or more members of a reducing sugar mixture *(4)* is necessarily low in accuracy.

The original Munson-Walker tables *(1)*, including Walker's table for maltose hydrate *(6)*, were established by direct weighing of the cuprous oxide precipitate. Since Hammond *(2)* showed the cuprous oxide to be contaminated with organic impurities, even when pure sugars were determined, his corrected table for glucose, valid for titrimetric determinations of reduced copper (see Jackson and McDonald *(7)*) is used here (Appendix II-A). Such corrected values for maltose hydrate are not available, therefore the data of Walker *(6)* are given in Appendix II-A. These values may be in error by about 1 percent when used with a titrimetric or electrolytic determination of copper. Accurate determinations of maltose hydrate should be based on a calibration by the operator

The original Munson Walker method *(1)* involved boiling over a gas flame and determining the precipitated cuprous oxide gravimetrically. The procedure presented here gives more uniform and reproducible heating and avoids inclusion of organic or inorganic impurities in the copper determination.

For gravimetric determinations the Cu_2O may be ignited to CuO, burning out organic impurities, and reduced to Cu by heating in an atmosphere of hydrogen. The sample may be weighed at any one of the three oxidation levels. The precipitated copper may also be dissolved in nitric acid and deposited electrolytically on a weighed platinum gauze electrode.

Volumetric determination of copper may be made by using thiosulfate or dichromate methods *(3b)*.

The end point of the permanganate titration may also be determined electrometrically, or an added indicator, ferrous phenanthroline solution (0.75 gm. of orthophenanthroline monohydrate in 25 ml. of 0.025 *M* ferrous sulfate), which gives a brown-to-green end point may be used. The indicator has not been favored by workers in the Starch and Dextrose Division of this Laboratory.

Acid ferric sulfate would dissolve cuprous oxide faster than does the neutral solution but would lead to low results, since some of the ferrous sulfate formed is oxidized by air under acid conditions.

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ADDITIONAL NOTES

B. SHAFFER AND HARTMANN MODIFICATION OF THE MUNSON AND WALKER METHOD

Introduction

The determination of copper by means of its reaction with iodide or iodine,

$$\text{I.}\quad 2Cu^{++} + 4I^{-} \longrightarrow 2CuI + I_2$$

$$\text{II.}\quad 2Cu^{++} + 2I^{-} \longleftarrow 2Cu^{+} + I_2,$$

has often been used in sugar determination methods. Shaffer and Hartmann *(1, 2)* have thoroughly studied the reactions and found means of getting them to go to completion so that they could be applied with greater accuracy to sugar determinations. They devised two analytical procedures for determining copper which they term the cupric titration (equation I) and the cuprous titration (equation II). In the cupric titration the equilibrium is shifted to the right by addition of a large excess of potassium iodide. The copper which was not reduced by the sugar is reduced by the iodide, and the liberated iodine is titrated with thiosulfate. However, since the precipitate of cuprous iodide interferes with the observation of the end point, most workers prefer the cuprous titration.

The equilibrium in the cuprous titration (equation II) is shifted completely to the left by removing the cupric ions as a cupric-oxalate complex. The iodine, liberated by acidification of iodate in the presence of iodide, oxidizes cuprous ions to cupric. The unused iodine is then titrated with thiosulfate.

Shaffer and Hartmann have employed the alkaline copper reagent and heating conditions specified by Munson and Walker *(3)* so that the tables worked out by the latter authors could be used. The Shaffer and Hartmann method has an advantage over other methods in that separation of the cuprous oxide from the reaction medium is unnecessary. Considerable saving of time for each determination is effected.

Reagents

Soxhlet solution "A" (copper sulfate).- 69.28 gm. cupric sulfate, $CuSO_4 \cdot 5H_2O$, per liter of solution (note 1). Filter through sintered glass. Determine the copper content (appendix I-C) and adjust to the correct concentration of 0.277 molar, or 440.9 mg. Cu in 25 ml. of solution.

Soxhlet solution "B" (alkaline tartrate).- 103.2 gm. sodium hydroxide, NaOH, reagent grade, 97 percent (note 2).
346 gm. Rochelle salt, $KNa(C_4H_4O_6) \cdot 4H_2O$
Dissolve ingredients in distilled water, make up to 1 liter and filter through sintered glass. The alkalinity of the solution should be determined by titration against standard acid. If the normality of the alkali differs from 2.50 *N* by more than two parts in 250, adjustment should be made. Somogyi *(5)* has shown that a small change in alkalinity may make a large change in reducing values.

Iodate-iodide solution.- 5.4 gm. potassium iodate, KIO_3.
60 gm. potassium iodide, KI
The salts are dissolved in water, 1 ml. of 3 *N* sodium hydroxide is added, and the solution made up to 1 liter. If this solution becomes yellow, it should not be used.

Sulfuric acid, 5 N.- 140 ml. of concentrated H_2SO_4 (sp. gr. 1.84) per liter.

Potassium oxalate, saturated solution.- Approximately 350 gm. $K_2C_2O_4 \cdot H_2O$ per liter will be required.

Standard sodium thiosulfate solution, 0.1 N.- See Appendix I-A.

Starch indicator solution.- See Appendix I-D.

Procedure

Introduce the approximately neutral sample into a 500 ml. Pyrex Erlenmeyer flask. Any volume of sample solution up to 50 ml. may be used, but if less is used, water must be added to bring the volume to 50 ±0.3 ml. The sample should not contain more than about 100 mg. glucose or 180 mg. maltose hydrate. Although tables are given for as little as 10 mg., the amount of sugar should be kept above 50 mg. glucose for accurate results.

Add 25 ml. of the copper solution (Soxhlet "A") from a pipet followed by 25 ml. of the alkaline tartrate solution (Soxhlet "B") carefully measured from a graduated cylinder. Add a pinch of talc, swirl, cover the mouth of the flask with a small beaker, and without delay place the flask on the electric heater (note 3). The heater must be adjusted (using a blank) so that the sample will come to a boil (note 4) in 4 minutes (±15 seconds). Boiling is continued for 2 minutes, giving a total heating period of exactly 6 minutes. Timing should be done with a stop watch. At the end of this period the flask is cooled immediately in a stream of cold water for 3 to 4 minutes. Care should be taken to avoid agitation of the reaction mixture up to the time that acid is added, otherwise, back oxidation of cuprous oxide by air will produce a low result.

From a precision pipet add 25 ml. of the iodate-iodide solution (note 5), and follow with 17 to 20 ml. of the 5 *N* sulfuric acid. The acid is measured with a graduated cylinder and is dumped rapidly into the flask (note 6). Cover the flask and swirl until all cuprous oxide is in solution. *This is important.* Next, add 20 ml. of the saturated potassium oxalate solution. Swirl until all of the cuprous iodide precipitate has dissolved (note 7). Titrate the excess iodine immediately with the standard 0.1 *N* thiosulfate. Run in thiosulfate until the green color becomes greenish blue, then add 2 to 3 ml. of the starch indicator, and titrate carefully until the dark blue of the starch-iodine complex disappears, leaving the light blue of the cupric oxalate complex. When the end point has been reached, one drop of the thiosulfate will not change the tint of the blue solution. A crystalline precipitate often appears near the end point but will not interfere.

In order to determine the total amount of iodine available under the conditions used, the blank titer must be determined. Twenty-five ml. of each of the Soxhlet solutions is mixed with 50 ml. of water and treated exactly as described for the sample. Care in delivering exactly the same volume of iodate-iodine solution each time is essential. Although the blank values for each lot of iodate solution will not change over a period of several months, it is customary to run a blank each day to check the regulation of the heater. Day-to-day differences observed in the boiling time are due apparently to changes in barometric pressure.

The equations on which this method is based are as follows:

$$\text{III.}\quad IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$\text{IV.}\quad Cu_2O + 2H^+ + I_2 \longrightarrow 2Cu^{++} + 2I^- + H_2O$$

$$\text{V.}\quad I_2 + 2S_2O_3^{--} \longrightarrow 2I^- + S_4O_6^{--}$$

To calculate the sugar content of the sample, subtract the volume of thiosulfate required for the sample from that required for the blank. The blank will usually require about 37 ml. of 0.1 *N* thiosulfate. The difference is the volume of standard thiosulfate equivalent to the iodine consumed by the copper reduced by the sample. Multiplying this volume of thiosulfate by the copper factor gives the weight, in milligrams of copper, reduced by the sample. The copper factor for 0.1000 *N* thiosulfate is 6.36 mg. per ml. (note 8). The weight of sugar equivalent to the weight of copper found is obtained from the proper column of the table, Appendix II-A. The result is milligrams of sugar in the sample and may be easily calculated to percent. If the sample is a solid, the results may be conveniently expressed as milligrams of maltose hydrate, or glucose, per gram of sample.

Notes

1. Most crystals or cupric sulfate are deficient in copper to the extent of 2 to 3 percent. This may be taken into account in making up the original solution.

2. Jackson and McDonald *(6)* point out that if the most accurate results are to be obtained, the alkaline carbonates will have to be removed from the sodium hydroxide because they lower the alkalinity. This is done by dissolving the sodium hydroxide at a 50 percent concentration, allowing the carbonates to settle out overnight, and filtering. The normality of this alkali will have to be determined and the proper amount used in making up the Soxhlet "B". This precaution usually is not taken in this Division, however, because for most purposes such accuracy in the result is not needed.

3. A rheostat controlled electric heater of open element type. Precision Scientific Company, Type PH, 550 watts is recommended. The heater should be turned on at least 30 minutes before the analyses are begun so that heating will be uniform.

4. It is rather difficult to establish exactly when boiling has begun. The following quotation from Jackson and McDonald *(6)* may be of help to the inexperienced worker: "....when a solution approached the boiling point, there usually occurred a few sporadic periods of apparent boiling which were followed by a very definite moment when the whole solution suddenly started to boil. This latter appearance was taken as the starting time of the 2-minute period."

5. Samples larger than 180 mg. maltose hydrate or 100 mg. glucose can be determined if 50 ml. of iodate-iodide solution is used

6. In alkaline iodine solutions, hypoiodite is formed which oxidizes the organic acids present. If the acid is not added rapidly, some iodine may be lost in this manner.

7. The precipitate of cuprous iodide will not be observed in most cases where only 25 ml. of iodate-iodide solution is needed.

8. If the thiosulfate is not exactly 0.1000 *N*, the factor can be found by multiplying the normality of the thiosulfate by 63.6.

Discussion

Apparently no direct comparison of accuracy has been made between the Shaffer and Hartmann method given here and those methods employing separation of the cuprous oxide. However, most workers believe that the latter methods are more accurate and that the Shaffer and Hartmann method sacrifices some accuracy for speed. For many purposes, such as the determination of sugar in enzymatic starch hydrolyzates and in other biological materials, the accuracy is satisfactory and the rapidity of determination is an asset.

The accuracy of the Shaffer and Hartmann method is dependent upon the amount of sugar present in the sample. For more than 50 mg. of glucose the accuracy is about 1 percent, and as the upper limit of sugar concentration is reached, the accuracy becomes greater. When 25 ml. of iodate-iodide solution is used, not more than about 100 mg. of glucose or 180 mg. of maltose hydrate can be determined.

This method suffers from a source of error common to many copper reduction methods; i.e., the back oxidation of cuprous oxide by air. Experience has shown that most workers using this method obtain low results. Such low results may be accounted for by actions which cause the precipitate of cuprous oxide to come into contact with oxygen. For instance, the sample may have contained dissolved oxygen due to aeration or rapid stirring; or agitation of the reaction mixture before the acid is added may cause contact between the particles of cuprous oxide and air. Considerable care must be exercised in handling the flask during the cooling and in adding the iodate-iodide solution, so that the surface of the liquid in the flask is disturbed as little as possible.

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ADDITIONAL NOTES

C. SOMOGYI METHOD FOR MICRO QUANTITIES OF REDUCING SUGARS

Introduction

The majority of reducing sugar methods require relatively large samples. Where conservation of materials is necessary, a procedure which can be used to determine very small quantities of sugars is essential. The reagent used in the micro method to be described is a "combined" copper reagent evolved by Somogyi *(1)*. The alkaline reagent is buffered with phosphates and includes potassium iodate as a source of iodine for the back oxidation of cuprous ion. The inclusion of 18 percent sodium sulfate is an innovation which Somogyi claims has virtually eliminated back oxidation of cuprous oxide by air. This has long been one of the main objections to copper methods. The present copper reagent was evolved from the original Shaffer and Hartmann combined reagent for determining small amounts of sugars *(2)*, as the result of a series of studies *(1,3,4,5)*.

The sugar samples are heated with the reagent in large test tubes in a boiling water bath. One advantage of this method is that the heating period does not have to be accurately timed. After the sample has been cooled, a solution of potassium iodide is added and the reaction mixture acidified to dissolve the cuprous oxide and release iodine from the iodate-iodide mixture. Any cuprous ion present is immediately oxidized by the iodine, and the excess iodine is then titrated with sodium thiosulfate solution. Each lot of copper reagent must be standardized against known quantities of pure sugars, an operation which constitutes the main disadvantage of the method. Precision of ± 2 percent is obtained for samples containing from 0.25 to 3.0 mg. of glucose or from 0.5 to 5.2 mg. of maltose hydrate. Smaller samples may be estimated, but with less accuracy.

Special Apparatus

Pyrex test tubes - 25 mm. O.D. x 200 mm.

Test-tube rack - This should be made of a stiff metal to hold test tubes in an upright position during heating. See Hagedorn and Jensen *(6)* for an illustration. A 4-liter stainless steel beaker (heated by a large hot plate) is convenient to use as a boiling water bath. A test-tube rack which will fit into this container (18 cm. diameter) can be designed with holes for as many as 17 test tubes, although 13-tube racks are usually employed.

Glass test tube closures. Glass thimbles, 15 mm. I.D., about 25 mm. deep, and with a flanged rim about 28 mm. O.D., have been found most convenient.

Glass stirring rods. Plunger type for the titration. Diameter of rod 5-6 mm.; length 19 cm.; handle 8-10 cm. long bent outward at a 45° angle; ring at bottom, 15-18 mm. O.D., the plane of which stands at right angles to the stem.

Reagents

Copper reagent. Directions for making 8 liters

32 gm. sodium hydroxide, NaOH
~~224~~ 565 gm. dibasic sodium phosphate, $Na_2HPO_4\ 12H_2O$
320 gm. Rochelle salts, $KNa(C_4H_4O_6)\ 4H_2O$
7.14 gm. potassium iodate, KIO_3
64 gm. cupric sulfate, $CuSO_4\ 5H_2O$ (note 1)
1 440 gm. sodium sulfate, anhydrous, Na_2SO_4

The sodium hydroxide, sodium phosphate, and Rochelle salts are dissolved in 500-600 ml. of distilled water with stirring and heating if necessary. It is best to dissolve the iodate in about 200 ml. of water with gentle heating, and then add to the main solution. The cupric sulfate crystals can be dissolved directly in the main solution or may be more conveniently added from a 10 percent stock solution (640 ml.). The sodium sulfate is

added last and dissolved (note 2). When solution is complete, the reagent is made up to 8 liters (graduated cylinder) and allowed to stand for 2 or 3 days during which time impurities will settle out. The clear solution is siphoned off and filtered through sintered glass (note 3).

Potassium iodide solution, 2.5 percent - 2.5 g. of potassium iodide, dissolved in water containing 3 drops of 3 *N* sodium hydroxide, is diluted to 100 ml. This solution should be checked for hypoiodite occasionally. A few ml., acidified, should remain colorless when a drop of starch indicator is added. Use of an old KI solution was found to cause rapid return of starch-iodide color, spoiling the endpoint.

Sodium thiosulfate, 0.110 N standard stock solution (note 4). - See Appendix I-A.

Sodium thiosulfate, 0.0055 N (note 5). - Fifty ml. (accurately delivered by pipet) of the stock 0.110 *N* thiosulfate solution and 2 ml. of 3 *N* sodium hydroxide are placed in a 1-liter volumetric flask and diluted to the mark. This solution should be made up fresh as needed. It will probably not change on standing for several days.

Starch indicator, 1 percent. - See Appendix I-D.

Phenol red indicator. - 100 mg. of phenol red dissolved in 28 ml. of 0.01 *N* NaOH and diluted to 250 ml. with water.

Procedure

The sample should have reducing power not greater than 3.0 mg. glucose or 5.2 mg. maltose-H_2O, with best results to be expected if at least 1.0 mg. of glucose or 1.6 mg. of maltose-H_2O is represented. Unknowns may have to be tried at several dilutions. If the sample is acid, extremely alkaline, or buffered it must be neutralized (note 6). Traces of chloroform or phenol in the sample will decompose during the heating period and interfere markedly with the titration. If alcohol is present, a similar amount should be used in the blank.

An aliquot of the sample solution, 5 ml. or less, water (5.0 ml. minus the volume of sample used), and 5 ml. of the copper reagent are pipetted into the large pyrex test tube (25 x 200 mm.) and thoroughly mixed (note 7). The tubes are closed with the glass thimbles (or other adequate devices) and placed in the test tube rack. Each sample should be run in duplicate. The rack of tubes is placed in a boiling water bath which has been filled to about 5 cm. above the level of the liquid in the tubes. The samples are allowed to remain in the boiling water for 10 minutes if glucose is the only sugar present, or 20 minutes if maltose is present (note 8). Three blanks (5 ml. water with 5 ml. of reagent) must be run with each lot of samples. At the end of the heating period the tubes are cooled by placing the rack of tubes in ice water or cold tap water (notes 9, 10).

For the titration 2 ml. of the 2.5 percent potassium iodide solution is added to the tubes without mixing with the contents (note 11) and followed immediately with 1.5 ml. of 2 *N* sulfuric acid (note 12) added rapidly (a fast-flowing buret is suitable) with simultaneous agitation (equation I p. 15). Tubes must stand for at least 5 minutes (note 13) with at least two agitations before titration. It is most important that all the cuprous oxide be dissolved before titration is begun. The iodine not reduced by the cuprous oxide is titrated with the 0.0055 *N* thiosulfate, mixing thoroughly by use of the stirring rods previously described. When the solution is light yellow, one or two drops of starch indicator and a like amount of phenol red solution is added, and titration is continued to the final disappearance of starch iodine blue. The yellow color provided by the phenol red is an excellent contrast for the last traces of blue according to some workers, although others prefer to omit the phenol red.

The blank titration will require about 22 ml. of the thiosulfate solution, while all samples which contain reducing sugars will require less (note 14). Dilution of the sample solutions to the same volume as the blank before the end point is reached is sometimes helpful.

The titer of the sample subtracted from the titer of the blank is the volume of thiosulfate equivalent to the copper reduced by the sample. Although 3 blanks are run, only those within 0.05 ml. of the mean should be used in the calculation.

Standardization of each new lot of reagent is carried out against pure samples of the sugar that is to be analyzed. National Bureau of Standards "Dextrose" and carefully recrystallized maltose hydrate are the standards most frequently used. In each case two solutions, 60 mg. and 25 mg. of glucose or 110 mg. and 55 mg. of maltose hydrate per 100 ml., will cover the desired range if appropriate aliquots (3, 4, and 5 ml. from the more concentrated solution and 1, 3, and 5 ml. from that less concentrated) are used. The standards should be run in quadruplicate.

Results are averaged (note 15) and plotted with "mg. sugar" as ordinate and "ml. of thiosulfate required" (blank titer minus sample titer) as abscissa. The points should fall on a straight line. The slope of this line, which is the number of mg. of sugar equivalent to each ml. of the 0.0055 *N* thiosulfate solution, is subsequently used to calculate sugar values from all analytical data obtained with this particular lot of copper reagent. For glucose the standard curve is straight to the origin and the factor can be used for calculations of results at any concentration. On the other hand, the maltose curve is linear only for samples containing more than 0.50 mg. maltose. When extrapolated below this value, the linear portion of the curve intersects the maltose axis slightly above the origin. The difference is frequently so small as to lack practical significance and may usually be ignored, in which case the line is drawn through the origin. If sufficiently large, this quantity (in mg. maltose·H_2O) must be added to any values obtained by use of the maltose factor. When samples which contain less than 0.50 mg. of maltose must be analyzed, the values should be taken directly from the standard curve. In this region the curve is closely approximated by drawing a straight line from the 0.50 mg. point to the origin.

The equations for the reactions involved in this method are the same as for the Shaffer and Hartmann method:

$$\text{I.}\quad IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$\text{II.}\quad Cu_2O + 2H^+ + I_2 \longrightarrow 2Cu^{++} + 2I^- + H_2O$$

$$\text{III.}\quad I_2 + 2S_2O_3^{--} \longrightarrow 2I^- + S_4O_6^{--}$$

Sample calculation of glucose content of a sample: If the blank required 22.30 ml. and the sample required 15.70 ml. of 0.0055 *N* thiosulfate; the difference, 6.60 ml., is the thiosulfate "required by the sample." If the glucose factor (slope) for this particular lot of reagent was 0.171 mg. glucose per ml. of 0.0055 *N* thiosulfate (note 16), then

$$0.171 \times 6.60 = 1.13 \text{ mg. glucose}$$

Sample calculation for maltose hydrate: I. Assuming (a) the maltose·H_2O factor = 0.290 mg. per ml. of 0.0055 *N* thiosulfate, (b) the linear portion passes through the origin, and (c) titration data the same as above, 0.290 x 6.60 = 1.91 mg. maltose·H_2O; II. Assuming (a) the maltose·H_2O factor = 0.280 mg. per ml. of thiosulfate, (b) the linear portion of maltose calibration curve intersects the maltose axis at + 0.07 mg., and (c) the same titration data as above (0.280 x 6.60) + 0.07 = 1.85 mg. + 0.07 mg. = 1.92 mg. maltose·H_2O.

Maltose hydrate values are converted to anhydrous maltose if multiplied by 0.950. Percent sugar in the original solution, assuming it had been diluted for neutralization or other reasons, may be calulated thus:

$$\frac{\text{mg. sugar found}}{\text{ml. aliquot analyzed}} \times \frac{\text{ml. after dilution}}{\text{ml. of original solutions}} \times \frac{100 \text{ ml.}}{1000 \text{ mg. per gm.}} = \text{gm. of sugar/100 ml}$$

Thus, if a 4.00 ml. aliquot, taken after 20 ml. of original maltose solution was neutralized and diluted to 50 ml., yielded 4.60 mg. of maltose H_2O

$$\frac{4.60}{4.00} \times \frac{50}{20} \times \frac{100}{1000} = 0.287\% \text{ of maltose hydrate in the original solution}$$

Notes

1. 65.3 g. of $CuSO_4 \cdot 5H_2O$ is usually used, because the crystals are about 2 percent deficient in copper.

2. Heating will not hasten solution of the sodium sulfate because it is less soluble at 100° than at 40°. Addition of nearly all of the water required in conjunction with rapid mechanical stirring will help.

3. This solution deposits some cuprous oxide on standing. For this reason it is best to include a sintered glass plug at some point in the dispensing system. Deposition of the oxide does not change the sugar factors.

4. It is very important that this stock thiosulfate solution retain its original titer over long periods of time. For recommended preservatives, see Appendix IA.

5. This normality was chosen so that the titer of the blank would be below 25 ml., permitting the use of a 25 ml buret.

6. For samples which are weakly acidic it has been found practical to neutralize with one or two drops of 0.1 *N* sodium hydroxide after they have been pipetted into the tubes. An indicator may be added if needed. Samples which are strongly acidic, alkaline, or buffered, will have to be neutralized to phenolphthalein end point with NaOH or H_2SO_4 before pipetting. Errors of the order of 4 percent occur when the solution is not properly neutralized.

7. When the reagents are pipetted into the tubes without mixing they tend to remain in layers even during the heating period. This of course gives low results.

8. Accurate timing is not necessary for this method because the times selected are located on flat portions of the rate curves for each sugar and because blanks are run with each set of samples. It is best to keep the boiling time close to that specified, however, if the timing is within 1 or 2 minutes the results will be acceptable. The blanks do not change after the first 10 minutes of heating. Somogyi *(1)* has shown that galactose and arabinose require the same heating period as maltose. If the method is to be used for the determination of sugars other than those mentioned a rate curve will have to be run to determine the proper heating period.

9. The temperature of the contents of the tubes should be about 30° at the time the iodide and acid additions are made. Somogyi *(3)* states that the oxidation of cuprous oxide by iodine may be incomplete in 5 to 10 minutes if the temperature is much below 30°.

10. If a delay in the procedure is necessary the tubes may at this point be allowed to stand at room temperature or below for several hours before titrating.

11. From the time the tubes are placed in the boiling water bath until the acid is added it is important that the tubes not be agitated in order to minimize reoxidation of cuprous oxide by air.

12. Some analysts use 5 ml of 0.75 *N* sulfuric acid.

13. All tubes may receive iodate at the same time, followed by acid. If this is done, it is important that the tubes be covered while standing. The covers should be washed so that any of the condensate adhering to them will be returned to the tubes before titration.

14. The results for samples which have a titer of less than 5.0 ml. of thiosulfate are of doubtful accuracy because the copper solution is near exhaustion.

15. Any erratic titration values (differing from the average by more than ±0.10 ml.) should be discarded.

16. The factors for both glucose and maltose show some variation with different reagents. For glucose, Somogyi *(1)* reported a factor of 0.149 and J. Corman of this Laboratory checks this figure closely (0.150 ±0.002) using 0.0050 *N* sodium thiosulfate. For the stronger thiosulfate (0.0055 *N*) prescribed in this method, the factors obtained by three different analysts fall in the range 0.156 to 0.167 for glucose and 0.285 to 0.300 for maltose hydrate.

Discussion

Somogyi's method has many advantages over other micro reducing-sugar methods. If the sugar content is kept within the specified limits, the results will be quite accurate. In the range of 3.00 to 0.25 mg. glucose or 5.0 to 0.50 mg. anhydrous maltose, the precision of the method averages ±0.01 mg. for both sugars, or about ±2 percent as an overall average. Within the range 0.25 to 0.01 mg. glucose or 0.50 to 0.05 mg. of maltose the precision will be about ±10 percent.

In addition to the precautions noted in the text, attention should be called to possible interfering substances. It is known that the presence of inorganic halides and nitrates will cause a depression of the reducing values *(3,4)*. The results with samples which contain quantities of such salts should be interpreted with care. The apparent cause of the error is that these salts tend to keep the cuprous oxide in solution and increase the amount of back oxidation. A fine precipitate in the sample, such as zinc hydroxide, will also cause an error due to back oxidation of cuprous oxide *(5)*.

Although non-carbohydrate reducing substances are not as readily oxidized by copper reagents as by ferricyanide, there may be samples in which such substances could cause considerable error. The correction necessitated by the presence of such extraneous substances can be found by determining the residual reducing power after all sugars have been fermented with baker's yeast *(7)*. If the sample contains polysaccharides, acid hydrolysis must precede fermentation.

Besides the advantages already cited for this particular copper reagent, the method has certain mechanical advantages. It is rapid, easy to use, and a large number of samples can be handled in a day. It is thus well adapted to routine analyses. For rapid, routine determinations using about one-tenth the amount of sample required in this procedure, a more recent method, based on a copper-carbonate reagent and employing colorimetric technique, may prove valuable *(8)*.

Although several attempts have been reported from other laboratories to apply this method to the determination of methylated reducing sugars, results have not been entirely satisfactory. However, the hypoiodite method of Willstätter and Schudel (Method III-A.) has been successfully adapted to this purpose.

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ADDITIONAL NOTES

C-a THE DIFFERENTIAL DETERMINATION OF GLUCOSE AND MALTOSE IN MIXTURES

Introduction

The determination of glucose and maltose is based on the use of Somogyi's micro method for determining reducing sugars, and on the selective, quantitative removal with bakers' yeast of only glucose, or both glucose and maltose, depending on time and pH. This method, particularly applicable to the analysis of starch hydrolyzate mixtures, was described by Stark and Somogyi *(1)* and was adapted for use in this Laboratory by S. A. Watson and J. Plate with subsequent modification by H. A. Davis. By use of this procedure, one can determine glucose and maltose separately in mixtures which may include other reducing carbohydrates not fermented under the conditions used. A further advantage is that only relatively small samples are required.

Reagents

Reducing Power Reagents - The necessary reagents, equipment, and calibrations for Somogyi's micro method are described in Section I-C.

Bakers' Yeast - This should be freshly purchased, although it has been found usable after 2 weeks storage in the refrigerator.

A 15 percent suspension of the yeast in distilled water (note 1) is adjusted to pH 5.0 (±0.2) with 0.02 *N* sulfuric acid and centrifuged. The supernatant is discarded and replaced by an equal volume of distilled water which has been similarly adjusted to pH 5.0. The yeast is resuspended (note 2) and again centrifuged, and the process is repeated for a total of at least three centrifugings and until the supernatant liquid is clear. All centrifuging is at full speed (International centrifuge, No. 1). The first two washings may be done in 250 ml. bottles, but thereafter all centrifuging in this analysis is done in 50 ml. celluloid tubes spun 5 minutes at full speed in the conical head. Two such tubes are used for each determination of a sugar mixture, and two others for determining the moisture content of each batch of washed yeast. At whatever point the yeast is added to the celluloid tubes, it must be in 30 ml. aliquots carefully measured (±0.5 ml.) from a single, well-stirred suspension. Once in the celluloid tubes, the yeast is not removed. The supernatant from the last wash may be left in the tubes if the yeast is to be used the next day--a convenient arrangement since the remainder of the determination takes nearly a full working day. If this is done, the tubes should be stored in the refrigerator and the yeast resuspended and centrifuged, discarding the supernatant just before use.

Buffer - A nearly saturated solution of sodium bicarbonate is adjusted with dilute sulfuric acid or sodium carbonate, as may be required, so that dilution with nine parts of water gives a pH value of 8.4 to 8.6.

Copper Sulfate - An approximately 0.3 *M* copper sulfate solution is prepared and standardized as directed in Appendix I-C.

Procedure

The carbohydrate mixture to be analyzed is made into a solution (note 9) of known volume and adequate reducing power (note 3), the latter determined by the Somogyi micro-method and recorded as milliliters of 0.0055 *N* thiosulfate per ml. of sample. No buffer (note 7) should be present. The pH is adjusted to 5.0 (with 0.02 *N* sulfuric acid) before the dilution (with pH 5.0 water) to definite volume. The solution is preserved by saturating it with toluene and storing it in the refrigerator.

After the yeast has been centrifuged and the last wash water poured off, excess moisture on the inner walls of the tubes is removed carefully with filter paper, not disturbing the yeast. A volumetric pipet is used to add a definite amount of sample solution (note 4) to one of the tubes. Next, 3.0 ml. of the bicarbonate buffer solution is added, followed by enough distilled water to make a total of 30.0 ml. of liquid added to the yeast paste in the tube. (Ml. sample aliquot + 3.0 ml. + ml. H_2O = 30.0 ml.). The tube is marked with the sample identification and a subscript Roman numeral, I. An equal volume of the sample solution is placed in a second tube (marked II) and enough water, previously adjusted to pH 5.0 with 0.02 *N* sulfuric acid, added to make 30.0 ml. (Sample aliquot + pH 5.0 water = 30.0 ml.). It is usually convenient to run two or three samples at once, making up two tubes from each in this manner.

When all additions have been made, a smooth glass stirring rod is placed in each tube and the contents carefully stirred, completely dispersing the yeast paste in the sugar solution. The tubes are at once placed in a water bath at 30°, the time is noted, and the yeast is stirred occasionally to keep it in suspension.

The dilution factor, resulting from the moisture in the yeast, is determined as soon as possible after the fermentations are started (note 8). The two celluloid tubes reserved for this determination (see preparation of yeast) are filled, each with 30.00 ml. of the standardized copper sulfate solution, and the yeast paste is dispersed as with the sugar samples. After the suspensions have stood 15 or more minutes with occasional stirring, the tubes are covered with rubber caps (note 5) and centrifuged. The supernatant solution from each is saved and analyzed to determine the change in copper sulfate molarity. Results from the two tubes should agree within 3 percent.

When the tubes containing yeast, sugar, and carbonate buffer (those marked I) have been in the water bath for 25 to 30 minutes they are withdrawn and the stirring rods removed. Covered with rubber caps (note 5), the tubes are centrifuged and the clear supernatants removed. The yeast cake is discarded. The pH of these solutions should be checked to make sure that proper reaction conditions were maintained. Two 5-ml. aliquots of each supernatant are promptly analyzed for reducing power by the Somogyi micro method (note 6). If these determinations cannot be run at once the solutions must be preserved under toluene in tightly stoppered flasks held in the refrigerator.

The remaining tubes, marked II, are similarly handled after being in the 30° bath for at least 2 hours.

Calculations

The dilution factor is the ratio of the molarity of the original copper sulfate solution to that of the solution diluted by yeast-moisture, or $\frac{[Cu]_1}{[Cu]_2}$. For example, if the original solution is 0.280 *M* and the centrifuged supernatant solutions average 0.260 *M*, the dilution factor is $\frac{0.280}{0.260} = 1.08$.

The reducing power remaining after each fermentation, first recorded as ml. of thiosulfate per 5 ml. of supernatant (r_I after the 30-minute, r_{II} after the 2-hour fermentation), is multiplied by six $\left(\frac{30\text{ ml.}}{5\text{ ml.}}\right)$ times the dilution factor, divided by the milliliters of original sample used in each tube. The results, R_I and R_{II}, represent the reducing power of 1 ml. of original sample from which, in one case, glucose, and, in the second, both glucose and maltose have been removed. The original reducing power of the sample is R_o. Glucose is calculated from $(R_o - R_I)$, maltose from $(R_I - R_{II})$, using the appropriate factor for conversion of ml. thiosulfate to mg. of glucose or maltose.

R_{II} may be used to express the remaining reducing power as equivalent maltose or to estimate the average molecular weight of the unfermentable dextrins if the dry weight of the sample is known.

Example. - Dry sample No. 1, 176.5 mg., dissolved and diluted to 50.00 ml., is designated 1_o. If the reducing power of original solution, Rl_o = 8.00 ml. of thiosulfate per ml., volume of original solution in each fermenting tube = 15.00 ml., and dilution factor = 1.08, then:

$$r_I = 12.00 \text{ ml.}, \quad R_I = 12.00 \times \frac{6 \times 1.08}{15} = 5.19 \text{ ml. of thio/ml. of original sample.}$$

$$r_{II} = 8.00 \text{ ml.}; \quad R_{II} = 8.00 \times \frac{6 \times 1.08}{15} = 3.46 \text{ ml. of thio/ml. of original sample.}$$

Reducing power factors. Glucose = 0.165 mg./ml. of thiosulfate.
Maltose·H_2O = 0.293 mg./ml. of thiosulfate.

Conversion of R values to glucose, maltose, and reducing dextrins

Composition of original solution						Dry sample
Thiosulfate reducing power (ml./ml.)	Conversion factor	Sugar concentration	Volume	Total sugar	Identity of sugar	Composition
		mg./ml.	*ml.*	*mg.*		*percent*
$R_o - R_I = 2.81$	0.165	0.464	50	23.2	Glucose	13.1
$R_I - R_{II} = 1.73$	0.293	0.506	50	25.3	Maltose·H_2O	14.3
$R_{II} = 3.46$	0.293	1.013	50	50.6	Reducing dextrins as maltose·H_2O	72.6

Reducing dextrins:

Weight: original sample - (glucose + maltose) = 176.5-(23.2 + 25.3) = 128.0 mg.

Milliequivalents: $\frac{50.6}{360} = 0.141$ m.e.

Molecular weight: $\frac{\text{Wt. in mg.}}{\text{m.e}} = \frac{128.0}{0.141} = 909$

Average chain length: $\frac{\text{Mol. wt.} - 18}{162} = 5.5$ glucose units

Notes

1. 15 gm. of pressed yeast suspended in 100 ml. of distilled water.

2. Resuspend centrifuged yeast with a glass stirring rod. Take particular care to avoid loss once the yeast has been measured into the celluloid tubes.

3. At least 6.0 ml. and not over 18.0 ml. of thiosulfate (1.7 to 5.4 mg. of maltose·H_2O; 0.9 to 3.0 mg. of glucose) should be represented by a 5-ml. aliquot of the solution in the celluloid tubes before any fermentation has taken place. The most dilute sample solution should therefore have reducing power equivalent to 1.44 ml. of thiosulfate (0.42 mg. maltose·H_2O or 0.24 mg. glucose) per ml., and at least 60 ml. of such a solution would be required (two 25-ml. portions for fermentation and two 5-ml. portions for the direct analysis). The practical upper limit of reducing power is about 18 ml. of thiosulfate per ml. of sample solution. About 14 ml. of such a solution would be used (6 ml. in each of the two fermenting tubes and two 1.00-ml. samples for the direct analysis).

4. The total amount of reducing sugars added to the yeast should be near the upper limit (equivalent to 108 ml. of thiosulfate, 17.8 mg. glucose or 31.6 mg. maltose·H_2O); the volume used should not exceed 25 ml.

5 Rubber caps prevent evaporation which could appreciably increase the concentration of the solutions being centrifuged.

6. Somogyi reducing powers should be recorded in terms of ml. of 0.0055 *N* thiosulfate per 5 ml. aliquot.

7. The presence of buffer in the sample solution at pH 5.0 would interfere with the adjustment to pH 8.4 for removing glucose and would also lower the alkalinity of the Somogyi reagent sufficiently to invalidate the reducing power determinations.

8. Yeast, a living substance, will change its physical characteristics on prolonged standing.

9. Any turbid material in the sample solution should be removed by centrifuging in the manner later used to remove yeast. Otherwise if the turbid material possesses any reducing power, this will be included in the total reducing power (R_0), but will be lost in removing yeast, causing the glucose result to be high.

Discussion

The consumption of glucose from standard solutions in the short fermentation period has sometimes been incomplete. For that reason, if particularly reliable results are desired, it may be well to repeat the determination on the unknown with a parallel determination of a known mixture having approximately the same composition.

The accuracy of the method also depends on that of the reducing power determinations and on the amounts of fermentable sugars present If R_0 and R_I, or R_I and R_{II} are very nearly equal, nominal deviations in the reducing power determination or the yeast dilution effect from tube to tube may cause relatively large changes in the amount of glucose or maltose indicated. Even a small negative result is sometimes obtained. However, the answers should be accurate within 0.25 mg. of maltose (or 0.15 mg of glucose) per ml. of original solution, and should improve in absolute (though not percentage) accuracy with the more dilute solutions. Where there is particular interest in the nature of the unfermentable reducing carbohydrates it may be valuable to parallel the Somogyi reducing power determinations with measurements by a micro version of one of the stoichiometric methods, such as that of Willstatter-Schudel or of Kiliani-Militzer. *(4, 5)*

The method used here for calculating the dilution of the samples by "free" moisture in the centrifuged yeast requires care in the addition of yeast slurry to the centrifuge tubes, but it avoids the weighings and extra calculations of the published method. Its accuracy, an important factor in the whole determination is as high as that of the more complex method, the probable variance being within 1 percent.

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ADDITIONAL NOTES

D. SCHOORL METHOD FOR DETERMINING REDUCING SUGARS IN DEXTRINS

Introduction

The method of Schoorl *(1, 2, 3, 4)* is a copper method employing Fehling solution. The reduced copper is determined indirectly by iodometric titration of the unreduced copper salt remaining after the sugar oxidation. The accuracy of the method has been vouched for by a number of eminent chemists, and tables are available *(3)* for the expression of results in terms of glucose, maltose, maltose hydrate, fructose, invert sugar, sucrose and lactose.

The method has been used at this Laboratory to determine the reducing power of pyrodextrins in terms of percentage glucose. Results have been consistent and the reproducibility good. In order to obtain an appreciable amount of reduced copper without using too large a sample, a known amount of glucose is added to each unknown and to each blank.

Reagents

Soxhlet solution "A" (cupric sulfate). - 69.28 gm. of pure cupric sulfate crystals, $CuSO_4 \cdot 5H_2O$, per liter of solution. (See p. 9 and note 1, p. 10)

Soxhlet solution "B" (alkaline tartrate). - 346 gm. of Rochelle salt, $KNaC_4H_4O_6 \cdot 4H_2O$, and 100 gm. of carbonate-free sodium hydroxide per liter of solution. (See note 12, p. 5 and note 2, p. 10)

Potassium iodide, 30 percent. - Dissolve 150 gm. of analytical reagent grade potassium iodide in water, add 1 ml. 3 *N* sodium hydroxide and dilute to 500 ml.

Sulfuric acid, 25 percent. - Add one volume of concentrated sulfuric acid (sp. gr. 1.84) to six volumes of distilled water.

Standard dextrose solution. - Weigh 1.000 g. of Bureau of Standards "Dextrose" into a 100 ml. volumetric flask and dilute to the mark.

Standard sodium thiosulfate, 0.1 N. - See appendix I-A.

Starch indicator solution. - See appendix I-D.

Procedure

Weigh 0.500 gm. (dry basis) of the dextrin to be analyzed into each of two 250 ml. Erlenmeyer flasks. Provide two additional flasks for the dextrose blanks. Into each flask, pipet in order

5.00 ml. standard dextrose solution
25 ml. distilled water
10 ml. Soxhlet solution "A"
10 ml. Soxhlet solution "B"

Mix well, and heat each flask on an electric heater with rheostat control (see method I-A, note 3). The heater must be adjusted to bring the solution to a boil in 3 minutes (I-A, notes 4 and 5, and I-B, note 4). Boiling is continued for 2 minutes, then the flask is cooled immediately under the cold water tap to room temperature. Ten ml. of the 30 percent potassium iodide solution is added, followed *(not preceded)* by 10 ml. of 25 percent sulfuric acid. The liberated iodine is titrated immediately with 0.1 *N*

standard sodium thiosulfate solution (dispensed from a 50 ml. buret) until the iodine color has become yellow. Soluble starch indicator solution (about 5 ml.) is now added and the titration is continued slowly until the blue color has disappeared and only the cream yellow shade of cuprous iodide remains. The blue color should not return for at least 1 minute.

The equations for the reactions involved are given in Appendix I-C. The difference in titers between the solution containing both dextrin and dextrose and the solution containing only dextrose is proportional to the reducing sugars in the dextrin sample; hence:

$$[\text{ml. } Na_2S_2O_3(\text{sample}) - \text{ml. } Na_2S_2O_3 \text{ (dextrose only)}] \times \frac{\text{normality } Na_2S_2O_3}{0.1000} =$$

ml. 0.1000 *N* $Na_2S_2O_3$

The percentage of glucose equivalent to the reducing sugars in the dextrin sample is read directly from the table in Appendix II-B. Duplicate samples should agree within 0.08 percent glucose in the range usually encountered (0.5 to 4.0 percent glucose).

The procedure may be checked by running 5.00 ml. of the 1.000-percent dextrose solution against a blank containing 30 ml. distilled water and the Soxhlet reagents. Difference in titers times the normality of the thiosulfate solution times 10 should equal 15.47 ml. of 0.1000 *N* $Na_2S_2O_3$, which is equivalent to 50.0 mg. glucose.

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ADDITIONAL NOTES

E. SICHERT AND BLEYER MODIFIED METHOD FOR DETERMINING "TRUE" DEXTROSE

(Reproduced by permission of the Corn Products Refining Company)

Introduction

Barfoed *(1)* formulated a copper acetate reagent that is not reduced appreciably by disaccharides. The reagent was adapted for quantitative use by Steinhoff *(2)*, who substituted sodium acetate for acetic acid and determined copper iodometrically. Sichert and Bleyer *(3)* have presented a detailed method of analysis for dextrose based on the use of Steinhoff solution. This method as modified by Peniston and Cantor (May 1941) of the Corn Products Refining Company for determination of their "true" dextrose is given here. It depends upon the selective oxidation of dextrose in a sodium acetate solution by cupric ion. The precipitated cuprous oxide is determined by dissolving it in ferric sulfate solution and titrating the resulting ferrous ion with ceric sulfate. The amount of dextrose present is then calculated from the titer by means of an equation, valid in the range 25-55 mg. of dextrose, or by use of a table calculated from the equation.

Reagents

Copper sulfate solution - Dissolve 72.7 gm. $CuSO_4 \cdot 5H_2O$ in water and dilute to 1 liter. Standardize as in method I.F, p. 30. (12.50 ml. equivalent to 125 mg. dextrose).

Sodium acetate solution - Dissolve 500 gm. $NaC_2H_3O_2 \cdot 3H_2O$ in water and dilute to 1 liter.

Ferric ammonium sulfate solution - Dissolve 135 gm. $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ in water and dilute to 1 liter. The ferric sulfate solution used in the Munson and Walker method may also be used.

N sulfuric acid - Slowly pour 28 ml. concentrated sulfuric acid into 900 ml. water. When cool, dilute to 1 liter.

0.1 N ceric sulfate - Dissolve by warming 53 gm. $Ce(HSO_4)_4$ (from G. Frederick Smith Chemical Co.) in a solution of 100 ml. concentrated sulfuric acid in 800 ml. water. Cool, filter through a sintered glass funnel, and make up to 1 liter.

Phosphoric acid, 50 percent - Dissolve 460 ml. 85 percent orthophosphoric acid (H_3PO_4) in water and dilute to 1 liter.

Diphenylamine indicator, 0.1 percent - Dissolve 0.46 gm. diphenylamine $(C_6H_5)_2NH$, in 250 ml. concentrated sulfuric acid.

Procedure

Into a 250 ml. Erlenmeyer flask are pipetted 10.00 ml. copper sulfate solution, 20 ml. sodium acetate solution, and 20.00 ml. (containing 25-55 mg. dextrose) of the sugar sample being analyzed. The flask is fitted with a Bunsen valve and is immersed in a calmly boiling water bath for exactly 20 minutes. The water level should be up to the neck of the flask, and the flask should be out of line with steam bubbles to avoid superheating. The precipitated cuprous oxide is collected by vacuum filtration on an asbestos mat supported in a filter funnel by a Witt plate placed over a glass "nail" (note 1). Quantitative transfer of the cuprous oxide is not necessary, but the flask and filter funnel should be rinsed with hot distilled water. This should be accomplished rapidly so that as little air as possible is drawn through the cuprous oxide. The filter funnel in its supporting rubber stopper is then withdrawn from the filter flask and

placed into the original Erlenmeyer flask. The glass nail thus lifts the Witt plate and mat in the filter funnel. About 20 ml. of hot ferric ammonium sulfate solution is slowly poured over the cuprous oxide, then the oxide and asbestos are rinsed through the filter funnel into the flask with 80 ml. of hot water. The mixture is stirred for a few seconds to aid solution and about 50 ml. of *N* sulfuric acid is added. The Bunsen valve is replaced and the flask is warmed on a hotplate with occasional stirring until solution of the cuprous oxide is complete (3 to 4 minutes). The flask is then cooled to room temperature in an ice bath or under a cold water tap, the Bunsen valve is removed, and the solution titrated with 0.1 *N* ceric sulfate solution. About 2 ml. before the end point is reached, 10 ml. of 50 percent phosphoric acid and 0.8 ml. 0.1 percent diphenylamine indicator are added. The solution is titrated to a definite purple color which should be stable for 1 minute.

The reactions involved in the stoichiometric relationships are:

$$Cu_2O + 2Fe^{+++} + 2H^{+} \longrightarrow 2Cu^{++} + 2Fe^{++} + H_2O$$

and

$$Ce^{++++} + Fe^{++} \longrightarrow Ce^{+++} + Fe^{+++}$$

The ceric sulfate solution is standardized against Bureau of Standards "Dextrose" by following the above procedure exactly with 20.00 ml. of 0.25 percent solution (0.6250 gm./250 ml.). For this amount of dextrose (50.00 mg.), 16.91 ml. of 0.1000 *N* ceric sulfate solution is necessary according to the equation determined for this method:

$$\text{Log mg. dextrose} = 0.94722 + (0.04448)\ (\text{ml. } 0.1000\ N \text{ ceric sulfate}).$$

The value obtained in the standardization will be different from 16.91 ml, because the ceric sulfate solution will not be exactly 0.1000 *N* and there will be differences in individual technique. To be able to use the equation, a "dextrose factor"

$$\frac{16.91}{\text{ml. ceric sulfate for 50.00 mg. dextrose}}$$

is calculated (note 2). Ceric sulfate titers obtained in unknown samples are then multiplied by this factor to convert them into ml. of 0.1000 *N* ceric sulfate. To determine mg. of dextrose, the above equation or a table (see Appendix II-C) calculated from this equation is used. The equation is valid between 25 and 55 mg. of dextrose only and must not be extrapolated beyond this range.

Notes

1. The filter funnel is made of a Pyrex No. 6220 filling funnel with the stem extended to 80 mm. in length. The Witt plate should be 25 mm. in diameter and have beveled edges to permit a firm fit with the accurate 60° angle of the molded funnel wall. The glass "nail" should be made from 5-mm. diameter glass rod, 23 cm. long, with a 15-mm. diameter head serrated on the edge to permit rapid filtering.

2. The Corn Products Refining Company method calls for the standardization of the ceric sulfate solution and its adjustment to 0.1000 *N* before determination of the "dextrose factor." The necessity for this is not seen.

Discussion

The above method has been found rather laborious and time-consuming, partly because it is difficult to get checks on duplicate samples. The range of the method is small, necessitating one or more preliminary determinations before the proper dilution for a sample is found. At best, the precision and accuracy are in the range of four to five parts per thousand. Maltose in large amounts has been found to interfere *(4)*.

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ADDITIONAL NOTES

F. CORN INDUSTRIES RESEARCH FOUNDATION METHOD FOR DETERMINING DEXTROSE EQUIVALENT

Introduction

The official analytical method of the Corn Industries Research Foundation for the determination of dextrose equivalent (note 1) is based upon the Lane and Eynon volumetric method *(1)* which is, according to Bates *(2)*, the "most convenient and frequently the most accurate method for the determination of reducing sugars." It differs from most reducing sugar methods in that the volume of sugar solution required to reduce a definite amount of alkaline copper reagent is determined. The end point for the reduction is internally indicated by the reduction of methylene blue to methylene white by an excess of the reducing sugar. Because methylene white is rapidly reoxidized by air, it is essential that air be excluded during the titration by uninterrupted boiling of the contents of the titration flask.

As the method is designed to determine dextrose equivalent in CSU (note 2) samples it is applicable only in the range 0.60 to 1.20 gm. dextrose in 100 ml. of solution.

Reagents

Soxhlet reagents - Copper sulfate solution. Dissolve 34.64 gm. of pure copper sulfate crystals ($CuSO_4 \cdot 5H_2O$) in water, and dilute to 500 ml.

Alkaline tartrate solution. Dissolve 173 gm. of Rochelle salt ($NaKC_4H_4O_6 \cdot 4H_2O$) and 50 gm. sodium hydroxide in water, and dilute to 500 ml.

Methylene blue indicator - One percent aqueous solution.

Procedure

The CSU sample is prepared for analysis by weighing out 15 gm. ($\pm$0.1 gm.) to the third decimal place, dissolving in water, and diluting to 500 ml. in a volumetric flask. The solution is poured into a standard buret (note 3) equipped with a glass outlet tube bent twice at right angles in order that the solution in the buret shall not be heated by the reaction mixture.

Twenty-five ml. of mixed Soxhlet reagent (note 4) which has previously been standardized against National Bureau of Standards "Dextrose" is pipetted into a 250-ml. Pyrex Erlenmeyer flask and brought to a boil over an open flame. Into the flask, which has been removed from the flame, is run almost all the solution prepared above, the amount being determined by preliminary titration (note 5) so that not more than 0.5 ml. is required later to complete the titration. The flask is again heated over the flame and rotated to swirl the contents. After boiling has begun, the liquid is kept in moderate ebullition for 2 minutes. The flask is then removed from the flame and two drops (0.1 ml.) of methylene blue indicator and two or three drops of the sugar solution are quickly added before boiling is resumed. The titration is then continued to the end point (note 6). The entire boiling period should not exceed 3 minutes. The titer should be in the range 10 to 20 ml. (note 7).

The mixed Soxhlet reagent is standardized by titration against a solution of dextrose prepared by dissolving 5.000 gm. of Bureau of Standards "Dextrose" dried at 100° for several hours, in water and making up to 500 ml. in a volumetric flask. Twenty-five ml. of the reagent require 12.02 ml. (0.1202 gm. dextrose) of the dextrose solution (note 4).

The amount of dextrose present in the weight of the CSU sample used for analysis can be calculated by

$$\frac{0.1202 \text{ gm. x } 500}{\text{Titer (in ml.)}} = \text{gm. dextrose in 500 ml.}$$

Division of this value by sample weight will give gm. dextrose per gm. CSU sirup. If a value only slightly different from 0.1202 gm. dextrose is required for standardization of the copper reagent, such value is used. If the departure from 0.1202 gm. is large, the copper solution should be adjusted.

Notes

1. Dextrose equivalent (D.E.) is defined as reducing sugars, calculated as dextrose, and expressed as a percentage of the dry substance. For the method of determining dry substance, see Appendix I-E.

2. CSU is an abbreviation for Corn Sirup Unmixed

3. The official method calls for a "special purity burette" of 20 ml. capacity, 0 to 10 ml. contained in a bulb and the portion 10 to 20 ml. graduated in 0.1 ml. Glass stopcocks give no special trouble if kept out of the steam.

4. The mixed Soxhlet reagent is prepared by measuring equal amounts of the two prepared solutions in a graduate and mixing. The solutions are the same as those used in the Munson and Walker method. Small departures in standardization from 12.02 ml. have not been found significant and are to be expected *(3,4)*. The mixed reagent is stable for several days at least.

5. The preliminary titration is carried out by adding about 8 ml. of the sugar solution to the measured volume of copper solution, boiling for about 15 to 20 seconds, and then adding further increments of sugar until the blue color of the copper solution has nearly disappeared. The methylene blue is added and the titration completed dropwise.

6. A white porcelain plate for background and a daylight lamp will assist materially in obtaining exact results.

7. In order to avoid the effect of concentration upon the amount of sugar required to reduce the copper completely, the method is limited to the range of 10 to 20 ml.

Discussion

The method as given is satisfactory for its intended purpose--the determination of reducing sugars in CSU samples, where the approximate amount of these sugars is known. A large number of determinations can be run rapidly and with the precision and accuracy of a good volumetric method A minimum of reagents is necessary and calculation of results is simple as no table, graph, or equation is used to relate titer to amount of copper reduced

As a general method for reducing sugars it suffers, with the unmodified Lane and Eynon method, from the fact that preliminary knowledge of the approximate sugar content of the unknown is necessary. The range of the Corn Industries Research Foundation method is also too limited for general use; the Lane and Eynon method is useful over a wider range of sugar values by the use of factors which take into account the effect of sugar concentration *(1,2)*.

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ADDITIONAL NOTES

II. FERRICYANIDE METHODS

A. BLISH AND SANDSTEDT METHOD

Introduction

In 1923 Hagedorn and Jensen *(1)* introduced a method for the micro determination of reducing sugar in blood. The method is based on the reduction of ferricyanide to ferrocyanide when heated in alkaline solution with reducing sugars. It possesses a major advantage over most copper reduction methods, because ferrocyanide is stable in air and no back-oxidation occurs. The precision of the method is excellent, and a series of analyses is more rapidly performed than is possible with most methods requiring Fehling solution. However, the oxidizing action of ferricyanide is not as specific as that of copper complexes, since it is more easily reduced by substances other than sugars. In common with the copper methods, the oxidation yields a variety of products, some of which undergo further oxidation; consequently, no stoichiometric relationship exists for the sugar oxidation.

Hanes *(2,3)* modified the method of Hagedorn and Jensen to determine up to 3 mg. glucose and 5 mg. maltose. He found it suitable for the estimation of maltose in the presence of soluble starch, when the slight reduction by the starch was taken into account. Factors for maltose, invert sugar, lactose, galactose, fructose, mannose, ribose, rhamnose, and xylose for use with the Hanes procedure are given by Hawk and Bergheim *(3)*.

Blish and Sandstedt *(4)* with collaborators in different laboratories found that the method was unsurpassed in reliability, simplicity, and convenience for the estimation of diastatic activity of flour. They increased the range of the method above that of Hanes. Sandstedt *(5)* further increased the range by using more concentrated (0.1 *N*) solutions. Kneen and Sandstedt *(6)* used the revision of Sandstedt to determine β-amylase activity and they prepared a table for the conversion of reduced ferricyanide to maltose equivalents. The work of Blish, Sandstedt, and Kneen is combined in the method described below.

This method is recommended primarily for the determination of the saccharifying activity of enzyme preparations *(6)*, particularly in the determination of β-amylase activity. It may be used for the rapid estimation of small amounts of maltose in hydrolyzates, especially when many determinations are to be made concurrently. The method will probably yield more consistent results than will copper reduction methods in the hands of inexperienced workers.

A critical survey on the determination of dextrose and maltose by the ferricyanide-carbonate reagent has been made by Lampitt, Fuller, Goldenberg, and Green *(7)*. They found that the oxidative power of the reagent is decreased by the presence of sodium chloride and sodium acetate in quantities such as would be present after acid hydrolysis of starch or with the use of an acetic acid-sodium acetate buffer.

Reagents

Alkaline ferricyanide solution, 0.1 N. - 32.9 gm. potassium ferricyanide, $K_3Fe(CN)_6$. 44.0 gm. anhydrous sodium carbonate, Na_2CO_3.

Dissolve in water and dilute to 1 liter. Lampitt, *et al.* *(7)* recommend that the ferricyanide and carbonate solutions be kept separately and mixed just before use, and that the ferricyanide solution be kept in an amber-colored bottle in the dark. If the carbonate and ferricyanide are mixed in advance, the solution should be allowed to stand 3 days and then it should be filtered before use. Store in the dark.

Zinc sulfate-potassium chloride-acetic acid solution.-

40 gm. zinc sulfate heptahydrate, $ZnSO_4 \cdot 7H_2O$
70 gm. potassium chloride, KCl
200 ml. glacial acetic acid, CH_3COOH
Dissolve in water and dilute to 1 liter.

Potassium iodide solution, 50 percent.- Fifty gm. of potassium iodide is dissolved in water, 3 drops of 3 *N* sodium or potassium hydroxide are added, and the solution is diluted to 100 ml. (note 1).

Sodium thiosulfate solution, 0.1 N.- See Appendix I-A.

Starch indicator solution, 1 percent.- See Appendix I-D.

Special Apparatus

Large Pyrex test tubes.- 29 mm. O.D. x 200 mm.

Test tube rack.- This may be a wire basket with loops for suspension in the bath, or the rack described for use in the Somogyi micro copper method.

A deep pan or bain-marie.- A stainless steel beaker large enough to hold sufficient water so that boiling will not stop when the tubes are introduced, is suitable.

Glass stirring rods.- Plunger type. Glass rod, 5-6 mm. diameter, is bent into a circular loop at the end, the plane of the loop being at a right angle to the stem. The diameter of the loop must be less than that of the test tubes so that the rods can be moved freely with vertical strokes.

Glass bulbs.- Sealed, 32-35 mm. diameter, for capping the tubes (optional).

Microburet.- 10 ml. capacity.

Procedure

All determinations should be run in duplicate. Five ml. of the sample is delivered with a precision pipet into a large Pyrex test tube. The sample should contain more than 2 mg. and less than 30 mg. of maltose hydrate or its equivalent. If less than 5 ml. of sample is taken, water must be added to make 5.0 ml. of sample (note 2). A blank is prepared by adding 5.0 ml. water to a similar tube (note 3). Ten ml. of the alkaline ferricyanide solution is added with a precision pipet to the sample and to the blank. No drops should remain on the side walls of the tube. With the water boiling vigorously and uniformly, the rack of tubes is immersed in the bath (note 4). The tubes are covered with glass bulbs or small watch glasses and are allowed to remain in the boiling water bath for exactly 20 minutes (note 5), then they are cooled by plunging the rack of tubes into a bath of crushed ice and water (note 6).

A plunger-type stirring rod is inserted in each tube, and, in turn, just before the titration, each mixture is acidified. Twenty-five ml. of the zinc sulfate-potassium chloride-acetic acid solution is taken in a graduate, but at first only a few ml. is added at a time with stirring, lest the escaping carbon dioxide cause loss by excessive foaming. The latter half of the acid solution may usually be poured with safety. There should be no delay between the acidification and titration *(8)*. One ml. of 50 percent potassium iodide solution is added and thoroughly mixed, then 2 to 3 ml. of the starch indicator is added (note 7). The mixture is constantly stirred while being titrated with the standard 0.1 *N* thiosulfate added from a 10 ml. microburet (note 8) until the blue or violet color has completely disappeared. The end point is very sharp since the opaque white precipitate present forms a contrasting background against which very small concentrations of the violet starch-iodine complex may be seen.

The equation for the sugar oxidation is not known. Ferricyanide is reduced to ferrocyanide in proportion to the amount of reducing sugar present. On acidification the carbonate is decomposed, and simultaneously the zinc and potassium ions in the solution precipitate the ferrocyanide as potassium zinc ferrocyanide.

$$CO_3^{--} + 2Fe(CN)_6^{----} + 2K^+ + 3Zn^{++} + 2H^+ \longrightarrow$$

$$CO_2 + H_2O + K_2Zn_3[Fe(CN)_6]_2$$

The ferricyanide not reduced by the sugar is reduced by the potassium iodide, and the equivalent iodine is liberated. The precipitation of the ferrocyanide in the reaction below as the potassium zinc salt allows the equilibrium to be displaced completely to the right

$$2\ Fe(CN)_6^{----} + 2I^- \rightleftharpoons 2Fe(CN)_6^{----} + I_2$$

The iodine liberated is titrated with thiosulfate.

$$I_2 + 2S_2O_3^{--} \longrightarrow 2I^- + S_4O_6^{--}$$

To calculate the maltose content of the sample, the volume of thiosulfate required for the sample is subtracted from that required by the blank. The net volume in ml. times the normality of the thiosulfate gives the milliequivalents of ferricyanide reduced. From the table of Kneen and Sandstedt *(6)* given in Appendix II-D, the corresponding amount of maltose (or maltose equivalents) in the sample may be found (see Discussion).

Notes

1 The alkali prevents the oxidation of iodide to free iodine. The solution should not be used if colored yellow. Sometimes traces of free iodine can be removed by filtering through two or more thicknesses of filter paper.

2 When an enzymatic hydrolysis is to be arrested at a specified time, it is convenient to add the sample of hydrolyzate (e.g., 3 ml.) to 1 percent sulfuric acid solution (2 ml.) in the test tube, the combined volume totalling 5.0 ml. In such case, the blank should contain the same amount of acid. The effect of this small amount of acid on the sugar and the analytical result has not been determined, but it is believed to be negligible.

3 If the sample contains degraded starch, the blank should contain preferably the same amount and kind of degraded starch. If this is not possible, soluble starch or β-amylase limit dextrin may be substituted.

4 The rack support must be adjusted so that the surface of the liquid in the tubes is 6 to 8 cm. below the surface of the boiling water

5. A timer with a warning bell is preferred. It should be checked against a good watch.

6. It has been determined *(8)* that delay after cooling and *before acidifying* has no effect on the results. Storage for 3 hours at 0° of the boiled, cooled, alkaline mixtures caused no change in titers. Delay in titrating *after acidifying* did affect the results.

7 In titrating the blanks it is wise to withhold the starch indicator until the end point is nearly reached

8 The microburet should deliver drops of very small volume If necessary the tip may be coated with paraffin to reduce the volume per drop and prevent creeping of the solution up the outside of the tip

Discussion

Although Kneen and Sandstedt's table is given (Appendix II-D) for calculating the results, it is strongly recommended that each operator standardize the method against pure samples of the sugar being determined and under the prevailing experimental conditions. The method here given is for the analysis of maltose, but any reducing sugar can be determined if a pure sample can be obtained for standardization. The data for samples of the pure sugar can be plotted on a large sheet of cross-sectioned paper, and the results of determinations on unknown samples can be read from the graph.

On pure maltose solutions of known content the results by this ferricyanide method, using the Kneen-Sandstedt conversion table, have been uniformly 4.2 to 7.5 percent high (14 determinations by 3 analysts). If the maltose equivalents obtained from the table were taken as *maltose hydrate*, instead of anhydrous maltose, then the results would be correct within 2 percent. Other evidence that the table gives high results when interpreted as anhydrous maltose is that after enzymic hydrolysis, more than 100 percent conversion of potato amylose to maltose has been calculated from the tables. It is recommended that the maltose equivalents found in the table be recorded as maltose hydrate, $C_{12}H_{22}O_{11} \cdot H_2O$. The factor for converting maltose hydrate into equivalent starch is 0.900.

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ADDITIONAL NOTES

B. SCHOCH AND JENSEN PROCEDURE FOR THE FERRICYANIDE NUMBER OF STARCHES AND DEXTRINS

Introduction

The reducing power of starches is difficult to determine because it is slight, and there is considerable difficulty in producing homogeneous dispersions of starches. If the starch is not dispersed before adding the oxidant, the reducing value obtained will depend on the physical state of the particles. This observation was made with the ferricyanide method of Farley and Hixon *(1)*, and its use has been discontinued. Schoch and Jensen[3] have dispersed their starches in hot water before adding the ferricyanide solution. The method of determining the ferrocyanide produced by the reduction is essentially the same as that of Blish and Sandstedt *(2)*, or Gore and Steele *(3)*. (See also the preceding ferricyanide method for sugars.) The method can be used on dextrins and modified starches to obtain a measure of the extent of hydrolysis.

Reagents

Alkaline ferricyanide solution, 0.05 N.-

16.5 gm. potassium ferricyanide, $K_3Fe(CN)_6$
22 gm. anhydrous sodium carbonate, Na_2CO_3
Dissolve in water and make up to 1 liter. The solution should stand 3 days before use. The precipitate formed on standing should be removed by filtration. If the solution is stored in a brown glass bottle away from sunlight, it maintains its strength for some time.

Zinc sulfate-potassium chloride-acetic acid solution -

20 gm. zinc sulfate heptahydrate, $ZnSO_4 \cdot 7H_2O$
70 gm. potassium chloride
200 ml. glacial acetic acid
Dissolve in water and make up to 1 liter. The salts may be weighed to the nearest gram.

Potassium iodide solution, 20 percent.- 200 gm. of potassium iodide per liter. A pellet of sodium or potassium hydroxide should be added to prevent the formation of free iodine.

Sodium thiosulfate solution, 0.05 N.- See Appendix I-A for preparation and standardization.

Starch indicator solution, 1 percent.- See Appendix I-D.

Procedure

The starch sample (200-250 mg. dry weight) (note 1) is placed in a clean, dry 8-oz. Pyrex nursing bottle. Twenty-five ml. of water is added, agitating to disperse the sample. The bottle is heated in a boiling water bath (note 2) for several minutes, with frequent agitation and swirling to complete the dispersion of the sample. No lumps should remain. The alkaline ferricyanide solution is then added from a 25-ml. precision pipet, the bottle is stoppered with a vented rubber cap and heated in a vigorously boiling water bath for exactly 15 minutes, with occasional agitation (note 3). The bottle is removed from the bath and immediately cooled under a stream of cold water,

[3] Unpublished procedure

60 ml. of zinc sulfate-potassium chloride-acetic acid reagent is added cautiously (to prevent excessive foaming), followed by thorough mixing; then 20 ml. of the 20-percent potassium iodide solution is added. These solutions may be added from graduated cylinders. The iodine liberated is titrated with 0.05 *N* thiosulfate until the blue color of the starch-iodine complex disappears (note 4). Blanks are run on 25 ml. of the alkaline ferricyanide reagent delivered from the same precision pipet used previously. Without heating (note 5), 25 ml. of water and 60 ml. of the zinc sulfate-potassium chloride-acetic acid reagent are mixed with the ferricyanide, and then 20 ml. of 20-percent potassium iodide solution is added. The solution is titrated as before, but with the addition of starch indicator before the end point.

The equation for the oxidation-reduction cannot be written because no stoichiometric relation is known. Ferricyanide is reduced to ferrocyanide in proportion to the reducing activity of the starch. Acetic acid neutralizes the carbonate and acidifies the solution, causing foam as carbon dioxide is liberated. At the same time, the zinc sulfate in the reagent precipitates the ferrocyanide formed by the reduction as the potassium zinc salt. The reactions that occur on addition of the acetic acid-zinc sulfate solution are given in the following equations:

$$Na_2CO_3 + 2HOAc \longrightarrow CO_2 + 2NaOAc + H_2O$$

$$2K_4Fe(CN)_6 + 3ZnSO_4 \longrightarrow \underline{K_2Zn_3[Fe(CN)_6]_2} + 3K_2SO_4$$

$$K_3Fe(CN)_6 + 3HOAc \rightleftharpoons H_3Fe(CN)_6 + 3KOAc$$

When potassium iodide is added to the acid solution the following equilibrium reaction takes place:

$$2H_3Fe(CN)_6 + 2HI \rightleftharpoons 2H_4Fe(CN)_6 + I_2$$

But, since zinc and potassium ions are present, the ferrocyanide is precipitated as the potassium-zinc salt and the reaction goes to completion, iodine being liberated equivalent to the amount of unreduced ferricyanide present.

$$2H_4Fe(CN)_6 + 3ZnSO_4 + 2KCl \longrightarrow K_2Zn_3[Fe(CN)_6]_2 + 3H_2SO_4 + 2HCl$$

The iodine liberated is titrated with thiosulfate:

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

The ferricyanide number is the number of milliliters of 0.1000 *N* thiosulfate (or ferricyanide) required per gram of starch (dry basis). It is calculated thus:

$$\text{Ferricyanide number} = \frac{(\text{ml. thio. for blank} - \text{ml. thio. for sample}) \times \text{normality thio.}}{\text{weight of sample on a dry basis} \times 0.1}$$

Notes

1. With the more soluble starches and dextrins, the sample size may be increased to 0.5 to 1.0 gm. without change in the ferricyanide number; that is, the relationship between thiosulfate titer and sample size is linear. With raw cornstarch, the relationship is linear only to 250 mg., probably because of the difficult dispersibility of the starch granule. If the sample is of a type which may retrograde or form difficultly soluble clots, as with some pregelatinized starches, it should be wetted with 2 to 3 ml. of benzene before water is added.

2. A steam pot may be substituted for the boiling water bath. For the oxidation, the bottle should be insulated from the bottom with a wide, flat cork, and a beaker should be inverted over the pot opening so that steam completely surrounds the nursing bottle. It may be necessary to extend the heating time slightly to obtain results equivalent to those obtained in a boiling water bath. The required time will be that which gives ferricyanide numbers of 239 and 303 ±3 for maltose hydrate and glucose, respectively (see last paragraph of discussion).

3. If the ferricyanide is exhausted during the heating period as indicated by complete loss of the yellow color, the determination should be repeated with a smaller starch sample. Exhaustion may occur with hydrolyzed or oxidized starches of high reducing value.

4. It is presumed that the starch sample will act as its own indicator. If an intense blue or purple color is not formed, starch indicator solution should be added before the end point. Toward the end of the titration, the persistence of insoluble black flecks of starch-iodine may obscure the end point. In such case, the bottle should be stoppered with the thumb and vigorously shaken, then the titration is completed dropwise, shaking vigorously between each addition of thiosulfate.

5. Schoch and Jensen do not heat their blanks. This is acceptable for routine analyses of starches and dextrins. Of course theoretically the blanks should be heated; but little is gained thereby, since the heated blank titers are virtually constant for a given set of reagents and vary little from the cold blank titer.

Discussion

While the alkali number is more sensitive as a qualitative test for starch hydrolysis, ferricyanide reduction is recommended whenever results are to be expressed on a quantitative basis. However, it is doubtful whether ferricyanide reduction measures absolute terminal aldehyde content, since the ferricyanide reducing values of glucose and maltose are not in stoichiometric relation. Despite this fact, the ferricyanide method is preferred to either the copper or hypoiodite methods, since it is influenced to a much lesser degree by variables, such as sample size, oxidation time, and concentration of ferricyanide. The effects of time of digestion and concentration of ferricyanide on the ferricyanide number of a recrystallized amylose are shown in the following table[3]

Time of oxidation	Ferricyanide number of amylose	
	5 ml. 0.05 *N* ferricyanide	25 ml. 0.05 *N* ferricyanide
Hours		
0.25	1.12	1.2
0.5	1.15	1.3
1.0	1.3	1.6
2.0	1.5	2.1
3.0	1.6	2.5

The ferricyanide numbers of maltose hydrate and glucose (60-75 mg.) are 239 and 303 ±3, respectively, by the method described. Hanes *(4)* found that the ratio of the volume of thiosulfate to the weight of sugar is constant for maltose hydrate but varies somewhat for glucose. From Hanes' data obtained on much smaller amounts of sugars (0.5-4 mg. glucose), the ferricyanide numbers of maltose hydrate and glucose were calculated as 229 and 297 ±7, respectively. Gore and Steele *(3)* reported a much higher ferricyanide number for maltose hydrate, 263. Typical values obtained

by Schoch and Jensen for a twice-recrystallized sample of butanol-precipitated corn amylose are: 1.15, 1.22, 1.21 ml. of 0.1 *N* thiosulfate per gram of amylose. In this Laboratory dextrins of high reducing power (7 to 20 percent dextrose equivalent) gave reducing values by this method that agreed with values by a copper reduction method (Scales, *5*).

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ADDITIONAL NOTES

III. IODOMETRIC METHODS

A. MODIFIED WILLSTÄTTER AND SCHUDEL METHOD FOR DETERMINATION OF ALDOSES

Introduction

Aldoses, under proper conditions, may be oxidized quantitatively to aldonic acids by an alkaline iodine solution. This method, generally referred to as the Willstätter-Schudel determination *(1)*, is applicable to the determination of aldoses alone or mixed with ketoses or other carbohydrates in samples free from other iodine-consuming compounds.

The dissolved sample is treated with an excess of dilute iodine and cautiously added sodium hydroxide. After a few minutes the solution is acidified and the unused iodine titrated with standard thiosulfate solution.

Reagents

Iodine solution, 0.05 N.- Dissolve 6.346 gm. of resublimed iodine in 100 ml. of 40 percent KI solution. Dilute to 1 liter. Store in dark.

Sodium thiosulfate solution 0.10 N. For preparation and standardization (note 1), see Appendix I-A.

Starch indicator solution. See Appendix I-D.

Sodium hydroxide solution 0.1 N.- Four gm. of alcohol-free NaOH per liter.

Hydrochloric acid 4 N.- Thirty-four ml. concentrated HCl (sp. gr. 1.18) per 100 ml. solution.

Procedure

For best results the sample should represent approximately 0.45 millimols of aldose (80 mg. of hexose; 67 mg. of pentose), although it may vary from 20 percent to 110 percent of that amount. Duplicate samples should be prepared. Ethanol, acetone, and other substances reacting with iodine (note 2) must be absent.

The sample, dissolved in 10 ml. of water, is placed in a 250-ml. glass-stoppered Erlenmeyer flask with a mark at the 100-ml. level. After measuring in 40.00 ml. of 0.05 *N* iodine solution (20-ml. volumetric pipet), 35 ml. of 0.1 *N* sodium hydroxide is added dropwise from a buret over a 3-minute period with constant swirling of the flask's contents. Slow regular addition of sodium hydroxide is especially important during the first minute. After the sides of the flask have been washed down with sufficient water to bring the solution to the 100-ml. level, the flask is stoppered and let stand at room temperature for 15 minutes.

The reaction mixture is acidified with 4 ml. of 4 *N* hydrochloric or sulfuric acid (graduated cylinder) and promptly titrated with standard 0.1 *N* thiosulfate, adding 1 ml. of starch indicator solution after the iodine color fades to yellow. The end point is reached when the blue starch-iodine color disappears (note 3). The titer should be between 10 and 17 ml. if the iodine and thiosulfate solutions are close to the indicated normalities (note 4). A blank is run by titrating 40.00 ml. of the iodine solution with the standard thiosulfate.

The reactions on which this method is based are:

Hypoiodite formation: $I_2 + 2OH^- \longrightarrow IO^- + I^- + H_2O$

Aldose oxidation: $RCHO + IO^- + OH^- \longrightarrow RCOO^- + I^- + H_2O$

Acidification: $IO^- + I^- + 2H^+ \longrightarrow I_2 + H_2O$

Titration: $I_2 + 2S_2O_3^{--} \longrightarrow 2I^- + S_4O_6^{--}$

Each mol of aldose oxidized requires two equivalents of iodine, so the calculation is:

$$[\text{Blank-titer (in ml.)}] \times \text{normality of thiosulfate} \times \frac{180}{2} = \text{mgs. of aldohexose}$$

The factor (180), which is the molecular weight of the sugars, becomes 150 for aldopentose, 342 for anhydrous maltose or lactose. In some cases the number of millimols of aldose is the information most desired and the factor is omitted. Otherwise, it may be desired to express aldose content in milligrams per gram,

$$\frac{\text{mgs. of aldose found}}{\text{wt. of sample in grams}}$$

or percent aldose,

$$\frac{\text{mgs. aldose found} \times 100}{\text{wt. of sample in grams} \times 1000}$$

Notes

1. Thiosulfate may also be standardized against pure dextrose by following this procedure.

2. $CH_3 \cdot CHOH-$ and $CH_3 \cdot CO-$ groups react with iodine in alkali, giving iodoform. Unsaturated compounds may also prove reactive. Other substances, not aldoses, that consume iodine to a slight extent are: Mannitol, glycerine, sodium lactate, sodium formate, and urea. Dextrins consume variable amounts that may be quite large.

3. "Dead-stop" end point or other electrometric titration methods may also be used.

4. Titers below 10 ml. suggest that the reducing power of the sample was underestimated; the result may be low. Unduly small differences between titer and blank lead to magnification of errors on subtraction. Too little aldose in the sample means more oxidation of ketose or other accompanying substances.

Discussion

The iodometric determination of aldoses has been the subject of much discussion and many varying procedures have been recommended. In contrast to the copper oxidations, the method is not empirical but rests on the stoichiometric relationships of the overall reaction,

$$RCHO + I_2 + 3NaOH \longrightarrow RCOONa + 2NaI + 2H_2O$$

and conditions are sought under which these relationships will be realized exactly. The procedure used here has been adapted from the methods of Hinton and Macara *(2)* and Lothrop and Holmes *(3)* who did not support Kline and Acree *(4)* in their claim of significant over-oxidation by excess iodine.

One source of error in the reaction is the conversion of hypoiodite to iodate ($3\ NaIO \longrightarrow NaIO_3 + 2NaI$) which does not oxidize aldose. Low results are obtained if too much hypoiodite is thus removed before the aldose has been completely oxidized. Aldoses (mannose, lyxose, idose) whose C_2 hydroxyls are trans instead of cis (by projection formula) to their ring oxygens react more slowly *(5)* and are therefore particularly sensitive to this error. The gradual addition of alkali keeps the hypoiodite concentration low but continuous favoring the oxidation of aldose rather than iodate formation. Thus, Lothrop and Holmes *(3)* found that when the alkali was run in at once, glucose gave 99.82 percent, mannose only 80.68 percent of the theoretical reduction. By adding the alkali uniformly over 2 minutes, the values became 100.03 percent for glucose and 99.35 percent for mannose.

The percentage oxidation of ketose samples by alkaline iodine is rather small (usually 1 to 4 percent) and is due principally to their conversion to aldose by the enediol rearrangement in the presence of alkali. Therefore, reaction time, temperature, and the amount of aldose originally present (hence net alkalinity, since alkali as well as iodine is consumed in oxidizing the aldose) affect the amount of ketose oxidation. Sucrose is only slightly oxidized, about 0.5 percent under the conditions of this procedure, even without appreciable aldose in the sample *(3)*. Sucrose oxidation does not increase nearly as rapidly with time and temperature as does ketose oxidation. Disaccharide aldoses give theoretical results *(2)* and those with longer chains probably do likewise. Methyl glucoses, such as those produced by the hydrolysis of methylated polysaccharides, may be determined with an error less than 2 percent *(6)*. A modification *(8)* of the Willstatter-Schudel method, capable of determining quantities of sugars of the order of 10 micrograms with a high degree of accuracy, has been applied to the estimation of sugars *(9)* and methylated sugars *(10)* separated on paper chromatograms.

When samples of optimum size are used, the method is accurate to within ± 0.5 percent on samples high in aldoses and, if run at 20°, may be combined with the Munson-Walker determination and the Lothrop-Holmes corrections *(3, 7)* for almost equally accurate determination of glucose and fructose in mixtures.

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ADDITIONAL NOTES

B CHLORAMINE-T METHOD FOR DETERMINATION OF ALDOSES

Introduction

To obtain quantitative oxidation of aldoses to aldonic acids with hypoiodite formed from iodine and caustic alkali, it is necessary that the concentrations of alkali and iodine be carefully controlled. The time allowed for the reaction and the manner of addition of the reagents are important factors. Over-oxidation of the sugar is possible, for under some conditions the primary hydroxyl group is slowly oxidized to a carboxyl group. Furthermore, if ketoses are present, excess alkali can cause rearrangement to aldoses which will be oxidized. By replacing the iodine solution with solutions of potassium iodide and Chloramine T (the sodium salt of N-chloro p-toluene sulfonamide, $[CH_3C_6H_4SO_2NCl]^- Na^+ 3H_2O$), many of the disadvantages listed above are removed. The Chloramine T hydrolyzes slowly, producing sodium hypochlorite, which reacts with potassium iodide to release the hypoiodite oxidizing agent at a slow rate. A very small amount of free iodine exists in the reaction mixture, and the reaction is run in very slightly alkaline solution. As a result, the oxidation proceeds more slowly than when strong alkali and iodine are used, the dangers of side reactions are minimized, and the reaction is more easily controlled. The Chloramine T method may be used wherever the iodometric method of Willstatter and Schudel is applicable.

Reagents

Chloramine T solution, 0.05 N. 7.1 gm per liter (the equivalent weight is one-half the molecular weight of the trihydrate, 281.70 ÷ 2 = 140.85).

Potassium iodide solution. 10 gm per 100 ml. Add a few drops of concentrated sodium or potassium hydroxide solution as a preservative.

Potassium hydroxide solution, approximately 0.1 N. 6 gm per 100 ml.

Sulfuric acid solution, 0.5 N. 14 ml concentrated H_2SO_4 (sp. gr. 1.84) per liter.

Sodium thiosulfate solution, 0.02 N, accurately standardized. For preparation and standardization see Appendix I A.

Starch indicator solution. 1 percent soluble starch solution, freshly made or well preserved; see Appendix I D.

Procedure

The sample should contain between 5 and 15 mg. of dextrose, or its equivalent. For larger amounts of dextrose (up to 80 mg.) the procedure outlined by Hinton and Macara (*1, 2*) should be followed (note 1). The sample may be dissolved in 1 to 10 ml. of water. A blank containing the same volume of distilled water used for dissolving the sample is treated exactly like the sample throughout the analysis.

To the sample contained in a 250-ml. Erlenmeyer flask (glass-stoppered preferred) is added in order:

50 ml. of distilled water from a graduated cylinder
2 ml. of 10 percent potassium iodide solution from a fast-flowing pipet.
1 ml. of 0.1 *N* potassium hydroxide solution from a fast-flowing pipet
10 ml. of Chloramine T solution accurately delivered from a precision pipet meeting Bureau of Standards specifications

The flask is stoppered, swirled, and allowed to stand for 90 minutes at 20° to 25° (note 2). The reaction mixture is then acidified with 5 ml. of 0.5 *N* sulfuric acid solution, or its equivalent, liberating iodine equivalent to the unaltered Chloramine-T and unused hypoiodite present. Iodine equivalent to 10 ml. of 0.05 *N* Chloramine-T solution is liberated from the blank (note 3); hence, approximately 25 ml. of 0.02 *N* thiosulfate will be required for the blank titration. For a 10 mg. dextrose sample the titer will be approximately 19 ml. The liberated iodine is titrated with 0.02 *N* standard thiosulfate solution contained in a buret (25 ml. buret preferred). As the thiosulfate is added, the iodine color becomes pale yellow. About 1 to 2 ml. before the end point is reached, 2 to 3 ml. of the starch indicator solution is added, and the titration with thiosulfate is continued to the disappearance of the blue color of the starch-iodine complex.

The equations for the reaction may be written as follows:

Hydrolysis of Chloramine-T to sodium hypochlorite:

$$C_7H_7SO_2NCl\cdot Na + H_2O \longrightarrow C_7H_7SO_2NH_2 + NaClO$$

Production of hypoiodite from hypochlorite and iodide:

$$NaClO + KI \longrightarrow NaCl + KIO$$

Oxidation of aldose to aldonic acid by hypoiodite:

$$KIO + RCHO \longrightarrow RCOOH + KI$$

Acidification:

a. $$2KI + C_7H_7SO_2NCl\cdot Na + H_2SO_4 \longrightarrow I_2 + C_7H_7SO_2NH_2 + NaCl + K_2SO_4$$

b. $$KIO + KI + H_2SO_4 \longrightarrow I_2 + K_2SO_4 + H_2O$$

Titration of iodine with thiosulfate:

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

Each molecule of aldose oxidized requires two equivalents of iodine; hence, the milliequivalent weight of dextrose is $\frac{180.16}{2} = 90.08$ mg. The weight of dextrose in milligrams found by the analysis is given by the product:

(ml. thio. for blank − ml. thio. for sample) x normality thio. x 90.08

The milliequivalent weight of anhydrous maltose is 171.15 mg., and for maltose hydrate or lactose hydrate 180.16 mg. If the sample taken was not a pure sugar, the reducing power may be expressed in percentage as equivalent dextrose by dividing the product above by the weight of the sample in milligrams and multiplying by 100.

Notes

1. The procedure given is that of Edwards, Nanji, and Chanmugam *(3)*, who adapted the method of Hinton and Macara for the analysis of small amounts of sugar found in dextrins. The method is scaled down by a factor of 5 from the original procedure.

2. The time of standing is not critical for pure sugars, nor does the temperature have much effect. A constant temperature of 17.5° was recommended by Hinton and Macara, but exact results have been obtained at 25° for 90 minutes. If the time of standing is

increased to 105 minutes at 25°, no significant changes are noted in the results. For pure dextrose in small amounts, the time may be reduced to 60 minutes at 25°.

3. The titer for the blank will decrease somewhat, since the Chloramine-T solution becomes weaker when the stock bottle is open. The solution should be kept in the dark when not in use.

Discussion

Dextrose, maltose, lactose, and invert sugar have been analyzed satisfactorily. The method has been used for the determination of lactose in fresh and condensed milk *(1)*; sucrose in the presence of lactose does not interfere. The method may be used also for mixtures of dextrose with smaller amounts of fructose. (See results below.) Substances which form iodoform with hypoiodite such as ethanol, acetone, acetaldehyde, isopropanol (or, in general, any compound containing the CH_3CO- radical, or one that may form it on oxidation), will affect the result. Pure methanol does not interfere.

The end point is very sharp, and the volumes of thiosulfate required for duplicate determinations should not vary by more than 0.04 ml. The factor of greatest importance in determining the precision obtained is the delivery of the Chloramine-T solution; exactly the same volume should be delivered to each flask.

Edwards, Nanji, and Chanmugam *(3)* used the method to determine glucose in pyrodextrins and maltose in enzymic dextrins, however, they did not prove that dextrins are not oxidized by the reagents. Additions of glucose and maltose to dextrins previously analyzed for the sugar resulted in accurate determinations of the amount of sugar added. Results obtained in this Laboratory show that the reducing power of pyrodextrins (no catalyst) is much higher by the Chloramine T method than by the Munson-Walker method. For example, on a series of wheat dextrins, the following results were obtained:

Temperature of dextrinization	Percent dextrose equivalent	
	By Chloramine T	By Munson-Walker
°C.		
175	5.9	1.5
185	7.2	1.8
195	11.1	2.8
200	13.8	3.8

Some results obtained by different operators on dextrose and maltose are given below.

	Dextrose	Dextrose	Dextrose	Maltose
Present (mg.)	5.16	7.81	10.1	18.0
Found (mg.)	5.23	7.84	10.2	18.8

Results obtained on levulose and mixtures of levulose and dextrose follow.

	Levulose	Levulose + dextrose	Levulose + dextrose
Present (mg.)	10.2	10.2 + 5.03	5.1 + 10.1
Found (mg. dextrose equivalent)	0.9	5.43	10.4

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ADDITIONAL NOTES

APPENDIX I

A PREPARATION AND STANDARDIZATION OF SODIUM THIOSULFATE SOLUTIONS

Preparation

Sterile non-acidic solutions of sodium thiosulfate are stable. If acids are present, even in small concentrations a cycle of reactions (equations I and II) proceeds continuously, and the concentration of thiosulfate is never constant.

$$\text{I} \quad H_2S_2O_3 \longrightarrow H_2SO_3 + S$$

$$\text{II} \quad H_2SO_3 + Na_2S_2O_3 \longrightarrow H_2S_2O_3 + Na_2SO_3$$

Carbonic acid may start this cycle, hence it is necessary to boil the distilled water used. Also boiling the water stabilizes the solution by sterilization, since thiobacteria can act on the thiosulfate *(1)*. It has been reported *(2)* that solutions made from boiled water do not require standardization more frequently than at monthly intervals.

Because sterile non-acidic conditions are desired, sodium thiosulfate solution should be stored in a stoppered narrow mouth bottle (2 gallons or 7.5 liters is a convenient capacity) fitted with a siphon tube and an air inlet containing a soda-lime tube and cotton. Calibrate the bottle by carefully pouring distilled water (7 liters) from a graduate, marking the level on the bottle. Boil the water plus an extra liter to allow for vaporization, in large Pyrex flasks for at least 15 minutes. Cool rapidly with inverted beakers over the flasks. While the water is boiling, the storage bottle and siphon tube may be sterilized with chromic acid cleaning solution. Transfer with a minimum of aeration the cooled boiled water (6 liters) to the well-rinsed bottle. Dissolve the required amount of clear sodium thiosulfate crystals, $Na_2S_2O_3 \cdot 5H_2O$ (24.82 gm. per liter of 0.1 *N* solution, 174 gm. for 7 liters, for 0.110 *N*, 27.30 gm. per liter) in the water and dilute with the cooled boiled water to the mark on the bottle. Stopper, mix thoroughly and let stand a day or two before standardizing.

Many preservatives have been recommended for thiosulfate solutions. Sterile solutions of pH above 6.0 need no preservative *(3, 4)*, but to insure protection of the solution over a period of time any one of these preservatives is recommended:

Sodium tetraborate (borax), $Na_2B_4O_7 \cdot 10H_2O$, 3.8 gm. per liter, pH 9.0-9.5 *(5)*.

Sodium tetraborate (borax) in the amount stated above plus 0.4 ml. chloroform per liter *(4)*.

A layer of toluene over the surface of the solution in the stock bottle.

Chloroform alone is not recommended unless the solution is stored in a dark brown rubber-stoppered bottle *(4)*.

Standardization

Primary standards most used for thiosulfate solutions are potassium dichromate, potassium iodate, copper, and iodine. Iodine is difficult to obtain sufficiently pure for this purpose (the A.C.S. grade of resublimed iodine is unsatisfactory), and possible losses by vaporization *(2)* make iodine inferior to the other three standards. The procedures are much simpler for standardization against potassium iodate and potassium

dichromate than for copper; hence, only the first two standards are recommended and presented here. They are non-hygroscopic solids, readily obtained in a pure state, and solutions of either are stable indefinitely.

Standardization against potassium iodate. Prepare a standard solution slightly weaker than 0.1 *N* by weighing approximately 3.55 gm. of A.C.S. quality potassium iodate (Baker's C.P. analyzed grade is satisfactory) accurately to the third decimal on an analytical balance, dissolving in distilled water, and diluting to exactly 1 liter in a volumetric flask. The transfer of iodate from the weighing glass to the volumetric flask is best made by placing a funnel in the mouth of the flask, inverting the glass in the funnel, and rinsing the weighing glass and funnel thoroughly with distilled water. A few drops of chlorobenzene should be added to prevent mold growth. Calculate the normality by dividing the exact weight of potassium iodate taken by the equivalent weight, 35.670 gm.

With a calibrated pipet, deliver precisely 50 ml. of the standard iodate solution (use 50 ml. for 0.1 *N* thiosulfate, 25 ml. for 0.05 *N*, and 10 ml. for 0.02 *N* thiosulfate) into each of two 250 ml. Erlenmeyer flasks. Add 2 gm. of potassium iodide (iodate-free) (note 2, Appendix I-C) and *just before the titration* add 10 ml. of *N* sulfuric or hydrochloric acid to liberate the iodine.

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

Titrate the liberated iodine immediately with the 0.1 *N* thiosulfate delivered from a 50-ml. buret. When the color has become a very pale yellow, 2 ml. of starch indicator (Appendix I-D) is added and the titration is continued dropwise to the disappearance of the blue color of the starch-iodine complex. The two titers must agree within 0.05 ml. Calculate the normality of the thiosulfate from the following expression:

$$\text{Normality thiosulfate} = \frac{\text{ml. standard iodate x normality iodate}}{\text{ml. thiosulfate required}}$$

Standardization against potassium dichromate (3). - Weigh accurately to the third decimal slightly less than 4.904 gm. (the weight required for 1 liter of 0.1000 *N* solution) of recrystallized A.C.S. (or Bureau of Standards) quality potassium dichromate. Dissolve in water and dilute to 1 liter in a volumetric flask. With a calibrated pipet deliver 50 ml. (for standardizing 0.1 *N* thiosulfate) of the standard dichromate into each of two 500-ml. Erlenmeyer flasks. For a blank determination 50 ml. water is delivered into a third flask. Add to each flask: 75 ml. water, 5 gm. of A.C.S. grade potassium iodide (iodate-free; note 2, Appendix I-C), and 5 ml. of 6 *N* hydrochloric or sulfuric acid with swirling. Wash down the sides of the flasks with a fine stream of water without agitation of the solutions, leaving a layer of water over the solutions of liberated iodine. Stopper the flasks and set in the dark for about 6 minutes. The reaction is

$$Cr_2O_7^{--} + 6I^- + 14H^+ \longrightarrow 3I_2 + 2Cr^{+++} + 7H_2O$$

Add water to a total volume of 300 ml., then titrate with the thiosulfate solution contained in a 50-ml. buret until the color becomes a light greenish-yellow. Add 2 ml. of starch indicator (Appendix I-D) and complete the titration to the disappearance of the blue-purple color of the starch-iodine complex. A clear blue-green color remains at the end point and is unaffected by further addition of thiosulfate. If any thiosulfate solution is required for the blank, the volume should be subtracted from the dichromate titers. The normality is calculated from the expression:

$$\text{Normality thiosulfate} = \frac{\text{ml. standard dichromate x normality dichromate}}{\text{net ml. thiosulfate required}}$$

A solution of 0.1 *N* thiosulfate was standardized by both methods, weighing each sample of iodate or dichromate individually. The normalities obtained were 0.1000, 0.1000, 0.1001, and 0.1003 against potassium iodate and 0.1001, 0.1002 and 0.1002 against potassium

dichromate. If only a single standardization is anticipated, samples of potassium iodate (0.13 to 0.17 gm.) or potassium dichromate (0.20 to 0.24 gm.) may be weighed individually. Since the normality of 0.1 *N* thiosulfate solution should be re-determined at intervals not exceeding 6 weeks, the preparation of a standard iodate or dichromate solution affords a great saving of time in the restandardizations. More dilute solutions of thiosulfate require more frequent standardizations.

References

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1923. The stability of sodium thiosulfate solutions. Jour. Amer. Chem. Soc. 45: 2132.

(2) Rice, F. O., Kilpatrick, M., Jr., and Lemkin, W.
1923. The estimation of iodine by sodium thiosulfate. Jour. Amer. Chem. Soc. 45: 1361.

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1942. Standardization and stability of 0.1 *N* sodium thiosulfate solutions in hot weather. Indus. and Engin. Chem., Anal. Ed. 14: 802.

(4) Kassner, J. L. and Kassner, E. E.
1940. Preparation of stable sodium thiosulfate solutions. Indus. and Engin. Chem., Anal. Ed. 12: 655.

(5) Watson, F. J.
1932. Preservation of standard thiosulfate. Soc. Chem. Indus. Victoria Proc. 32: 679; Chem. Abs. 27: 476.

ADDITIONAL NOTES

B. STANDARDIZATION OF POTASSIUM PERMANGANATE SOLUTIONS

It has been shown *(1)* that the normalities of permanganate solutions obtained with arsenious oxide and with sodium oxalate agree within 0.3 part per 1,000. The procedure using arsenious oxide is simpler in that no heating is required. Arsenious acid is quantitatively oxidized by permanganate in the presence of a trace of iodide ion.

For standardizing 0.15 *N* potassium permanganate solution, about 0.3 gm. of primary standard grade arsenious oxide, As_2O_3, is accurately weighed and transferred to a 400-ml. beaker (for .033 *N* permanganate, 0.1 gm. As_2O_3 is used). Ten ml. of cool 20-percent sodium hydroxide solution is added to dissolve the oxide. The clear arsenite solution is then diluted with 100 ml. water and acidified with 10 ml. concentrated hydrochloric acid. One drop of 0.0025 *M* potassium iodide or iodate is added, and the solution is titrated with 0.15 *N* potassium permanganate. The last 1 to 1.5 ml. of permanganate should be added dropwise, waiting for the solution to become clear before each drop is added. When the pink color persists for 30 seconds after the last addition, the end point has been reached. An electrometric end point or ferrous orthophenanthroline indicator may also be used.

A blank is run on all reagents except the arsenious oxide. Determination should be made in duplicate or triplicate.

$$\text{Normality } KMnO_4 = \frac{\text{Wt. } As_2O_3 \text{ in grams}}{0.04946 \times \text{net vol. } KMnO_4 \text{ (titer - blank)}}$$

Reference

(1) Bright, H. A.
1937. Arsenious oxide in the standardization of solutions of potassium permanganate. Indus. and Engin. Chem., Anal. Ed. 9: 577.

ADDITIONAL NOTES

C STANDARDIZATION OF COPPER SULFATE SOLUTIONS

The iodometric determination of copper is convenient and accurate The best features of the methods given in the three textbooks consulted *(1 2 3)* were combined in the directions given below

Ten ml (precision pipet) of the copper solution to be analyzed is transferred to a 125 ml Erlenmeyer flask Add 1 gm potassium acid phthalate (note 1) and 15 to 20 ml of water heat until the phthalate is dissolved Cool add 3 gm potassium iodide (note 2) and dissolve Cover the flask and allow to stand 2 or 3 minutes Titrate the liberated iodine with standard 0 1 *N* sodium thiosulfate (Appendix I A) to a pale yellow color When the titration has proceeded to within 0 5 ml of the end point (note 3), add 2 gm potassium or ammonium thiocyanate dissolved in 10 ml of water (note 4) and swirl for a few seconds Add 1 ml of starch indicator (Appendix I D) and continue the titration a drop at a time to the complete disappearance of the blue color (note 5) The equations for the reactions involved are

$$2Cu^{++} + 4I^{-} \longrightarrow Cu_2I_2 + I_2$$

$$I_2 + 2S_2O_3^{--} \longrightarrow 2I^{-} + S_4O_6^{--}$$

$$\text{Molarity copper sulfate} = \frac{\text{Normality thiosulfate x ml thiosulfate}}{\text{ml copper sulfate}}$$

Notes

1 The potassium acid phthalate will buffer the solution at pH 4

2 The potassium iodide used must be free of iodate To test for the presence of iodate proceed as follows Dissolve 0 1 to 0 2 gm of the potassium iodide in 1 to 2 ml of water acidify with a drop of 6 *N* sulfuric acid and add starch solution Immediate appearance of a blue color shows the presence of iodate Slow formation of color is caused by atmospheric oxidation

3 It is convenient to run a trial titration in which thiocyanate need not be used to determine the approximate titer of the sample

4 Cuprous thiocyanate is more insoluble than cuprous iodide The thiocyanate is added to remove cuprous ions more completely and to allow the reaction to go to completion

5 The precipitate of cuprous iodide will make observation of the end point somewhat difficult to the inexperienced worker, especially since the precipitate is often pink The pink color which is due to adsorption of iodine should be ignored in judging the end point Electrometric titration eliminates this difficulty

References

(1) Kolthoff I M and Sandell E B
1936 Textbook of quantitative analysis p 604 New York

(2) Pierce W C and Haenisch E L
1940 Quantitative analysis Ed 2 p 212 New York

(3) Willard H H and Furman N H
1933 Quantitative analysis p 196 New York

ADDITIONAL NOTES

D PREPARATION OF STARCH INDICATOR

Starch pastes must be sterilized or treated with a preservative to prevent degradation by micro organisms If starch indicator is required over a long period the preservative recommended is 30 percent sodium chloride if needed for only a week or two, a few ml of chloroform chlorobenzene or toluene may be added (after cooling) instead of the salt

Dissolve 150 gm of sodium chloride in 480 ml water in a large beaker and heat to boiling Stir vigorously with a motor-driven stirrer, and add slowly a smooth suspension of 5.7 gm. (approximately 5.0 gm dry weight) of soluble starch in 20 ml water Boil for at least 5 minutes and cool

The indicator is best dispensed from a dropping bottle (50 ml capacity), that is, a bottle containing a ground in pipet capped with a rubber bulb Whenever starch indicator gives a reddish purple color with iodine (especially noticeable just before the end point is reached in a titration), it should be discarded The color should remain deep blue purple until completely discharged

E DETERMINATION OF DRY SUBSTANCE IN CORN SIRUP (CSU) SAMPLES

The sirup is dried on filter aid in a vacuum oven after preliminary drying in an air oven In this Laboratory, a modification of the corn wet-milling industry method is used.

The CSU sample is prepared for analysis by weighing out 25 grams to the third decimal, dissolving in water and diluting to 250 ml in a volumetric flask Ten or 15 ml. of this solution (containing about 1 gm dry solids) is pipetted onto 7.5 or 12.0 gm., respectively of Johns Manville Hyflo Super Cel filter aid (note 1), contained in an aluminum dish The dish should be about 3 inches in diameter 1.5 inches deep, and have a tight-fitting lid The mixture is worked into a *damp* mass with a glass rod flattened at one end and short enough to fit inside the closed dish The dish rod and filter aid must have been weighed before adding the sample The determination is run in duplicate together with two blanks

The dishes, with covers about half off, are dried in an air oven at 70° to 75° for 6 to 15 hours or until the bulk of the moisture has been removed and the dried material is in a friable condition The glass rods are used to work the material to a powder and the dishes are transferred to a vacuum oven kept at 80° ±1° and 30 inches of vacuum for 30 hours At the end of this time the heat is turned off, but the vacuum is left on until the dishes are cool They are transferred to a desiccator and subsequently weighed to determine the amount of dry solids present The weight of dry substance per gram of CSU sirup is calculated from the average of the two determinations corrected for the blank

Notes

1 The filter aid is washed for use by percolation with distilled water slightly acidified with hydrochloric acid This treatment is continued until the effluent is acid to litmus The filter aid is then washed with distilled water until the effluent is essentially neutral air dried and then dried at 105°

ADDITIONAL NOTES

APPENDIX II

A. DEXTROSE AND MALTOSE VALUES FOR THE MUNSON AND WALKER METHOD

The values for dextrose given in this table are those re-determined by Hammond (Jour. Res. Natl. Bur. Standards 24: 589. 1940). Since the values given for maltose hydrate were determined by Walker (Jour. Amer. Chem. Soc. 29: 541. 1907) by weighing the cuprous oxide precipitate, they are not strictly applicable to quantities of reduced copper determined in other ways. See discussion on p. 5. Used by permission.

Copper	Dextrose	Maltose H_2O	Copper	Dextrose	Maltose H_2O
mg	*mg*	*mg*	*mg*	*mg*	*mg*
10	4.6	7.2	40	19.2	35.5
11	5.1	8.2	41	19.7	36.4
12	5.6	9.1	42	20.1	37.3
13	6.0	10.1	43	20.6	38.3
14	6.5	11.0	44	21.1	39.2
15	7.0	11.9	45	21.6	40.1
16	7.5	12.9	46	22.1	41.0
17	8.0	13.8	47	22.6	42.0
18	8.5	14.8	48	23.1	42.9
19	8.9	15.7	49	23.6	43.8
20	9.4	16.7	50	24.1	44.8
21	9.9	17.6	51	24.6	45.7
22	10.4	18.5	52	25.1	46.7
23	10.9	19.5	53	25.6	47.6
24	11.4	20.4	54	26.1	48.6
25	11.9	21.4	55	26.5	49.5
26	12.3	22.3	56	27.0	50.4
27	12.8	23.2	57	27.5	51.4
28	13.3	24.2	58	28.0	52.3
29	13.8	25.1	59	28.5	53.2
30	14.3	26.0	60	29.0	54.2
31	14.8	27.0	61	29.5	55.2
32	15.3	27.9	62	30.0	56.1
33	15.7	28.9	63	30.5	57.1
34	16.2	29.8	64	31.0	58.0
35	16.7	30.8	65	31.5	58.9
36	17.2	31.7	66	32.0	59.9
37	17.7	32.7	67	32.5	60.8
38	18.2	33.6	68	33.0	61.8
39	18.7	34.5	69	33.5	62.7

Copper	Dextrose	Maltose·H_2O	Copper	Dextrose	Maltose·H_2O
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
70	34.0	63.7	110	54.1	101.3
71	34.5	64.6	111	54.6	102.2
72	35.0	65.5	112	55.1	103.1
73	35.5	66.5	113	55.6	104.1
74	36.0	67.4	114	56.1	105.0
75	36.5	68.4	115	56.7	106.0
76	37.0	69.3	116	57.2	106.9
77	37.5	70.2	117	57.7	107.8
78	38.0	71.2	118	58.2	108.8
79	38.5	72.1	119	58.7	109.7
80	39.0	73.1	120	59.2	110.6
81	39.5	74.0	121	59.7	111.6
82	40.0	74.9	122	60.2	112.5
83	40.5	75.9	123	60.7	113.5
84	41.0	76.8	124	61.3	114.4
85	41.5	77.8	125	61.8	115.3
86	42.0	78.7	126	62.3	116.3
87	42.5	79.6	127	62.8	117.2
88	43.0	80.6	128	63.3	118.1
89	43.5	81.5	129	63.8	119.1
90	44.0	82.5	130	64.3	120.0
91	44.5	83.4	131	64.9	120.9
92	45.0	84.3	132	65.4	121.9
93	45.5	85.3	133	65.9	122.8
94	46.0	86.2	134	66.4	123.8
95	46.5	87.2	135	66.9	124.7
96	47.0	88.1	136	67.4	125.6
97	47.5	89.0	137	68.0	126.6
98	48.0	90.0	138	68.5	127.5
99	48.5	90.9	139	69.0	128.4
100	49.0	91.8	140	69.5	129.4
101	49.5	92.8	141	70.0	130.3
102	50.0	93.7	142	70.5	131.3
103	50.6	94.7	143	71.1	132.2
104	51.1	95.6	144	71.6	133.1
105	51.6	96.6	145	72.1	134.1
106	52.1	97.5	146	72.6	135.0
107	52.6	98.4	147	73.1	135.9
108	53.1	99.4	148	73.7	136.9
109	53.6	100.3	149	74.2	137.8

Copper	Dextrose	Maltose·H_2O	Copper	Dextrose	Maltose·H_2O
mg.	mg.	mg.	mg.	mg	mg.
150	74.7	138 8	190	95.7	176.3
151	75.2	139.7	191	96.3	177.2
152	75.7	140.6	192	96.8	178.1
153	76.3	141.6	193	97.3	179.1
154	76.8	142.5	194	97.9	180.0
155	77.3	143.4	195	98.4	180.9
156	77 8	144.4	196	98.9	181.9
157	78.3	145.3	197	99.5	182.8
158	78 9	146.3	198	100.0	183.8
159	79.4	147.2	199	100.5	184.7
160	79.9	148 1	200	101.1	185.6
161	80 4	149 1	201	101.6	186.6
162	81.0	150.0	202	102.2	187.5
163	81 5	150.9	203	102.7	188 5
164	82.0	151 9	204	103.2	189.4
165	82.5	152.8	205	103 8	190.4
166	83.1	153.8	206	104 3	191.3
167	83 6	154.7	207	104.8	192.2
168	84.1	155.6	208	105 4	193.1
169	84.6	156.6	209	105 9	194.1
170	85.2	157 5	210	106.5	195.0
171	85.7	158 4	211	107 0	195.9
172	86.2	159 4	212	107.5	196.9
173	86.7	160.3	213	108 1	197.8
174	87.3	161.3	214	108 6	198.8
175	87.8	162 2	215	109.2	199.7
176	88 3	163 1	216	109.7	200.6
177	88.9	164.1	217	110.2	201.6
178	89.4	165 0	218	110 8	202.5
179	89.9	166 0	219	111 3	203.5
180	90 4	166 9	220	111.9	204.4
181	91.0	167 8	221	112 4	205.3
182	91 5	168.8	222	112 9	206.3
183	92.0	169.7	223	113.5	207 2
184	92.6	170.6	224	114 0	208.1
185	93.1	171.6	225	114.6	209 1
186	93 6	172.5	226	115 1	210 0
187	94.2	173.4	227	115.7	211 0
188	94.7	174.4	228	116 2	211 9
189	95.2	175.3	229	116.7	212 8

Copper	Dextrose	Maltose·H_2O
mg.	*mg.*	*mg.*
230	117.3	213.8
231	117.8	214.7
232	118.4	215.6
233	118.9	216.6
234	119.5	217.5
235	120.0	218.5
236	120.6	219.4
237	121.1	220.3
238	121.7	221.3
239	122.2	222.2
240	122.7	223.1
241	123.3	224.1
242	123.8	225.0
243	124.4	226.0
244	124.9	226.9
245	125.5	227.8
246	126.0	228.8
247	126.6	229.7
248	127.1	230.7
249	127.7	231.6
250	128.2	232.5
251	128.8	233.5
252	129.3	234.4
253	129.9	235.3
254	130.4	236.3
255	131.0	237.2
256	131.6	238.2
257	132.1	239.1
258	132.7	240.0
259	133.2	240.9
260	133.8	241.9
261	134.3	242.8
262	134.9	243.7
263	135.4	244.7
264	136.0	245.6
265	136.5	246.6
266	137.1	247.5
267	137.7	248.4
268	138.2	249.4
269	138.8	250.3

Copper	Dextrose	Maltose·H_2O
mg	*mg*	*mg*
270	139.3	251.2
271	139.9	252.2
272	140.4	253.1
273	141.0	254.1
274	141.6	255.0
275	142.1	255.9
276	142.7	256.9
277	143.2	257.8
278	143.8	258.8
279	144.4	259.7
280	144.9	260.6
281	145.5	261.5
282	146.0	262.5
283	146.6	263.4
284	147.2	264.4
285	147.7	265.3
286	148.3	266.2
287	148.8	267.2
288	149.4	268.1
289	150.0	269.0
290	150.5	270.0
291	151.1	270.9
292	151.7	271.9
293	152.2	272.8
294	152.8	273.7
295	153.4	274.7
296	153.9	275.6
297	154.5	276.5
298	155.1	277.5
299	155.6	278.4
300	156.2	279.3
301	156.8	280.3
302	157.3	281.2
303	157.9	282.1
304	158.5	283.1
305	159.0	284.0
306	159.6	284.9
307	160.2	285.9
308	160.7	286.8
309	161.3	287.8

Copper	Dextrose	Maltose·H_2O	Copper	Dextrose	Maltose·H_2O
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
310	161.9	288.7	350	185.0	326.1
311	162.5	289.6	351	185.6	327.0
312	163.0	290.6	352	186.2	328.0
313	163.6	291.5	353	186.8	328.9
314	164.2	292.4	354	187.3	329.9
315	164.7	293.4	355	187.9	330.8
316	165.3	294.3	356	188.5	331.7
317	165.9	295.2	357	189.1	332.7
318	166.5	296.2	358	189.7	333.6
319	167.0	297.1	359	190.3	334.5
320	167.6	298.0	360	190.9	335.5
321	168.2	299.0	361	191.5	336.4
322	168.8	299.9	362	192.0	337.3
323	169.3	300.9	363	192.6	338.3
324	169.9	301.8	364	193.2	339.2
325	170.5	302.7	365	193.8	340.2
326	171.1	303.6	366	194.4	341.1
327	171.6	304.6	367	195.0	342.0
328	172.2	305.5	368	195.6	343.0
329	172.8	306.5	369	196.2	343.9
330	173.4	307.4	370	196.8	344.8
331	173.9	308.3	371	197.4	345.8
332	174.5	309.3	372	198.0	346.7
333	175.1	310.2	373	198.5	347.6
334	175.7	311.2	374	199.1	348.5
335	176.3	312.1	375	199.7	349.5
336	176.8	313.0	376	200.3	350.5
337	177.4	314.0	377	200.9	351.4
338	178.0	314.9	378	201.5	352.3
339	178.6	315.8	379	202.1	353.2
340	179.2	316.8	380	202.7	354.2
341	179.7	317.7	381	203.3	355.1
342	180.3	318.6	382	203.9	356.0
343	180.9	319.6	383	204.5	357.0
344	181.5	320.5	384	205.1	357.9
345	182.1	321.4	385	205.7	358.9
346	182.7	322.4	386	206.3	359.8
347	183.2	323.3	387	206.9	360.7
348	183.8	324.3	388	207.5	361.7
349	184.4	325.2	389	208.1	362.6

Copper	Dextrose	Maltose·H_2O
mg.	*mg.*	*mg.*
390	208.7	363.5
391	209.3	364.5
392	209.9	365.4
393	210.5	366.3
394	211.1	367.3
395	211.7	368.2
396	212.3	369.1
397	212.9	370.1
398	213.5	371.0
399	214.1	371.9
400	214.7	372.9
401	215.3	373.8
402	215.9	374.7
403	216.5	375.7
404	217.1	376.6
405	217.8	377.6
406	218.4	378.5
407	219.0	379.4
408	219.6	380.3
409	220.2	381.3
410	220.8	382.2
411	221.4	383.1
412	222.0	384.1
413	222.6	385.0
414	223.3	386.0

Copper	Dextrose	Maltose·H_2O
mg.	*mg.*	*mg.*
415	223.9	386.9
416	224.5	387.8
417	225.1	388.7
418	225.7	389.7
419	226.3	390.6
420	227.0	391.6
421	227.6	392.5
422	228.2	393.4
423	228.8	394.4
424	229.5	395.3
425	230.1	396.2
426	230.7	397.1
427	231.4	398.1
428	232.0	399.0
429	232.7	400.0
430	233.3	400.9
431	234.0	401.8
432	234.7	402.7
433	235.3	403.7
434	236.1	404.6
435	236.9	405.6

B. TABLE FOR REDUCING SUGARS IN DEXTRINS DETERMINED BY THE SCHOORL METHOD

Schoorl Conversion Table

(Interpolated from copyrighted table in Cereal Chemistry 10:474. 1933. Permission for publication has been granted.)

Ml. 0.1000 *N* $Na_2S_2O_3$	Percent glucose equivalent	Ml. 0.1000 *N* $Na_2S_2O_3$	Percent glucose equivalent
1.0	0.64	5.5	3.52
1.1	0.70	5.6	3.58
1.2	0.76	5.7	3.64
1.3	0.82	5.8	3.70
1.4	0.88	5.9	3.78
1.5	0.96	6.0	3.84
1.6	1.02	6.1	3.90
1.7	1.08	6.2	3.96
1.8	1.14	6.3	4.04
1.9	1.20	6.4	4.10
2.0	1.26	6.5	4.16
2.1	1.32	6.6	4.22
2.2	1.38	6.7	4.28
2.3	1.44	6.8	4.36
2.4	1.50	6.9	4.42
2.5	1.58	7.0	4.48
2.6	1.64	7.1	4.54
2.7	1.70	7.2	4.60
2.8	1.76	7.3	4.68
2.9	1.82	7.4	4.74
3.0	1.88	7.5	4.80
3.1	1.94	7.6	4.86
3.2	2.00	7.7	4.92
3.3	2.08	7.8	5.00
3.4	2.14	7.9	5.06
3.5	2.20	8.0	5.12
3.6	2.26	8.1	5.18
3.7	2.32	8.2	5.26
3.8	2.40	8.3	5.32
3.9	2.46	8.4	5.38
4.0	2.52	8.5	5.46
4.1	2.58	8.6	5.52
4.2	2.66	8.7	5.58
4.3	2.72	8.8	5.64
4.4	2.78	8.9	5.72
4.5	2.86	9.0	5.78
4.6	2.92	9.1	5.84
4.7	2.98	9.2	5.92
4.8	3.04	9.3	5.98
4.9	3.12	9.4	6.06
5.0	3.18	9.5	6.12
5.1	3.24	9.6	6.18
5.2	3.32	9.7	6.26
5.3	3.38	9.8	6.32
5.4	3.44	9.9	6.40
		10.0	6.46

C. TABLE FOR THE DETERMINATION OF "TRUE" DEXTROSE BY THE MODIFIED SICHERT AND BLEYER METHOD

Milliliters 0.1 *N* ceric sulfate to milligrams dextrose (calculated from the equation on p. 28)

Ml. 0.1 *N* $Ce(HSO_4)_4$	Milligrams dextrose									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
10	24.66	24.92	25.17	25.43	25.69	25.96	26.22	26.49	26.77	27.04
11	27.32	27.60	27.89	28.17	28.46	28.76	29.05	29.35	29.65	29.96
12	30.27	30.58	30.89	31.21	31.53	31.86	32.19	32.52	32.85	33.19
13	33.53	33.88	34.23	34.58	34.93	35.29	35.66	36.03	36.40	36.77
14	37.15	37.53	37.92	38.31	38.70	39.10	39.50	39.91	40.32	40.74
15	41.15	41.58	42.01	42.44	42.88	43.32	43.76	44.21	44.67	45.13
16	45.59	46.06	46.54	47.02	47.50	47.99	48.48	48.98	49.49	50.00
17	50.51	51.03	51.56	52.09	52.62	53.16	53.71	54.26	54.82	55.39

D. KNEEN AND SANDSTEDT'S TABLE OF MALTOSE EQUIVALENTS FOR BLISH AND SANDSTEDT'S FERRICYANIDE METHOD

Method of Blish and Sandstedt modified for 0.1 *N* reagents. Table is reproduced from Cereal Chemistry 18: 240. 1941. Used by permission.

0.10 *N* Ferricyanide reduced	Maltose*
ml.	*mg.*
0.50	1.46
1.00	2.92
1.50	4.38
2.00	5.85
2.50	7.32
3.00	8.79
3.50	10.28
4.00	11.83
4.50	13.40
5.00	14.97
5.50	16.57
6.00	18.20
6.50	19.85
7.00	21.50
7.50	23.15
8.00	24.88
8.50	26.70
9.00	28.65
9.50	30.65

*Interpreted as maltose H_2O. See second paragraph under Discussion, p. 36.